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SEPTEMBER, 1928

THE EFFECT OF INADEQUATE VITAMIN B UPON SEXUAL PHYSIOLOGY IN THE MALE[1]

HERBERT M. EVANS

(Department of Anatomy, University of California, Berkeley, Cal.)

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FOR some years the literature of nutrition has carried positive statements as to the deleterious effects on sexual physiology in birds and mammals caused by the withdrawal of the vitamin B[2] from a diet. As regards avian physiology, this question is still *sub judica*. In many cases a profound degeneration of the testis has been attributed to this deficiency. The recent admirable papers of Mattill[3] and his colleagues have, however, called into question, if they have not completely disproved, this contention as regards mammals. By reason of the precision with which our tests have been conducted as well as the great number of such tests, the present paper will establish, we hope, the immunity of the male germinal epithelium from the changes—some of them remarkable ones—characterizing the tissues of the body after the withdrawal of vitamin B. Long before the appearance of recent papers on this subject, this laboratory began its concern with the possible relations of vitamins B and E in male as well as female sterility. As Mattill has recently emphasized, almost none of the researches claiming degeneration of the testis as

[1] Aided by grants from the Committee for Research in Problems of Sex of the National Research Council and from the Bureau of Dairying of the United States Department of Agriculture. These funds have been generously augmented by the Board of Research and by the College of Agriculture of this institution.

[2] Throughout this paper the old designation vitamin B is used to cover the complex of two or more factors now known to be present in yeast. It is clear, however, that our experiments have involved chiefly a delinquency in the antineuritic rather than the purely growth promoting or pellagra preventing B, for we have shown that simplified diets containing casein, cornstarch and lard are usually sufficiently rich in the pure growth B to get along well when all yeast is withdrawn from the diet, providing a rather pure antineuritic extract (Tiki-tiki) is added. (Evans, H. M. and Burr, G. O. On a new differentiation between the antineuritic and purely growth-promoting water soluble vitamin. J. B. C., 1928.) Our removal of yeast undoubtedly lowered the growth (P. P.) vitamin, but the animals declined and died from the much graver lowering of the antineuritic component.

a result of lowered vitamin B have been conducted with the assured presence of vitamin E—the substance essential for normality of the testis. In fact the need of E by the male was unknown at the time all but the most recent researches were done.[4] Inasmuch as the most potent source of E known to us (wheat germ oil) need not be contaminated even with traces of vitamin B, a clear answer to this problem seems possible. In synthetic dietaries we have only to withdraw B, keeping an assured high level of E. The experiment may be conducted either by feeding from the beginning inadequate sources of vitamin B along with an abundance of E, or by the sudden withdrawal of B from adult animals properly nourished and continuing to receive a nutrition adequate in every respect and especially in respect to E, suffering merely delinquency in B. Very definite advantages attend an experiment of the first sort. It is quite conceivable that the almost precipitate death provoked by sudden and complete withdrawal of B, while actually disadvantageous to the testis, could not have time to show this particular relation because of its effect on other and more vital organs. The long continuance of life on an inadequate level of B would appear to give maximum opportunity for disadvantageous effects to disclose themselves in all the tissues of the body. Particularly also as regards the phenomena of behavior, the provocation of less rapidly fatal beri beri confers advantages, for we have the opportunity to test sexual activity with several oestrous females over a considerable period of time; fewer tests of this sort can be made after the complete withdrawal of B.

Both types of experiment have been carefully conducted by us; the first type—the conveyance from the day of weaning of an inadequate level of antineuritic B[5]—has been performed (without essential difference in the result) with two dietaries, our diets 55 and 228, the compositions of which are as follows:

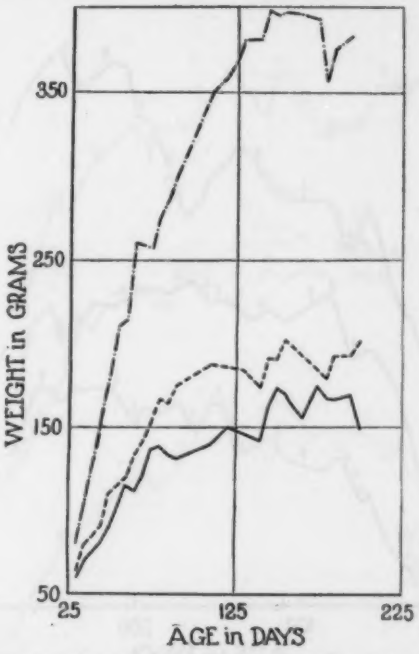
Diet 55		Diet 228	
Casein (extracted)	18	Casein (commercial)	27.
Cornstarch	54	Cornstarch	59.3
Lard	22	Lard	7.7
Cod liver oil	2	Cod liver oil	2.
Salts	4	Salts	4.
Yeast 0.5–0.7 gram daily		Yeast 0.5–0.7 gram daily	

[3] Mattill, H. A. The Relation of Vitamins B and E to Fertility in the Male Rat. *Amer. Jour. Physiol.*, 1927, vol. 79, p. 305.

[4] Hence the otherwise careful paper of Parkes and Drummond (The Effect of Vitamin B Deficiency on Reproduction; *Proc. Roy. Soc. B.*, vol. 98, pp. 147–171, 1925) is invalidated.

[5] We have shown that a related simplified diet (Diet 232: casein 32, cornstarch, cooked, 40, salts 4, lard 22, cod liver oil 2) is sufficiently rich in the purely growth promoting vitamin B

As regards Diet 55, the experiments were done with three groups of animals, three males in each group taken from the day of weaning (the twenty-first day of life) and placed on the ration to which was added daily in one group 0.1 gram of whole dried yeast, in the second group 0.2

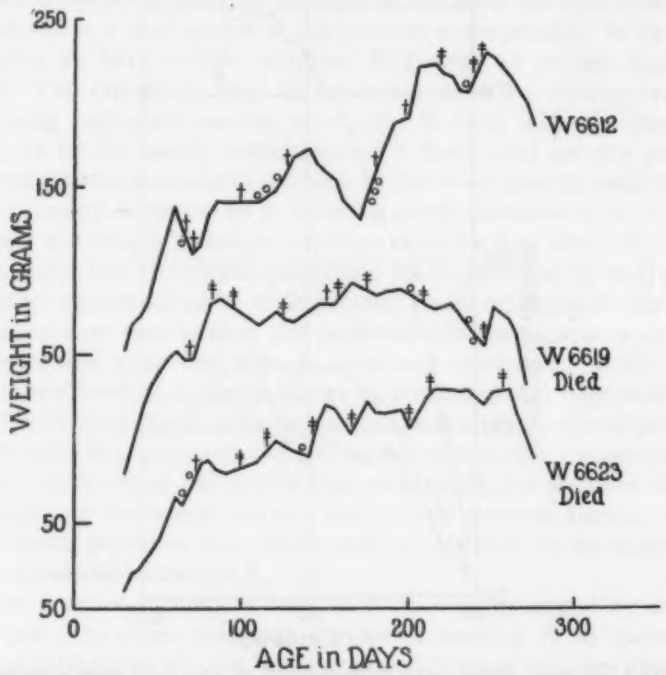


GRAPH 1. Composite growth curves of males reared on Diet 55+6 drops wheat germ oil and varying amounts of yeast—
———— 0.1 gm. yeast;
– – – – – – 0.2 gm. yeast;
—•—•—•— 0.8 gm. yeast.

gram daily, and in the third group 0.8 gram daily. All nine animals received daily on their yeast dose, six drops of wheat germ oil, adequate in all cases, as our fertility and histological tests show, to retain normality of the reproductive function. The animals were maintained in individual

(P.P.) to enable animals to undertake almost normal growth without yeast, provided an adequate amount of a relatively pure antineuritic vitamin (extract of TIKITIKI) is added. (Evans, H. M., and Burr, G. O. On a new differentiation between the antineuritic and purely growth-promoting water-soluble vitamin. *J. B. C.*, 1928.) It is important to note that the removal of yeast hence *to some extent* also lowers the growth promoting (P.P.) vitamin B though the animals undoubtedly declined rapidly and died from lack of antineuritic B.

cages with wire bottoms in order to avoid the unfortunate variations which ensue in such experiments if access to feces is allowed. On or shortly after the sixtieth day of life there was introduced into the individual male's cage a young, vigorous female which had been reared on natural food,



GRAPH 2. Individual curves of males reared on Diet 55+6 drops wheat germ oil+0.1 gm. yeast daily.

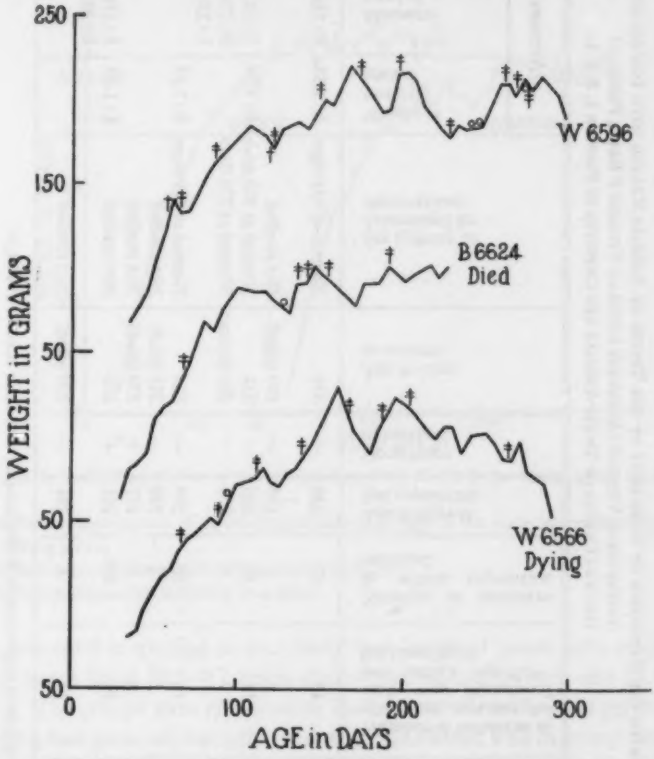
° indicates introduction of an oestrous female into cage of the male but without copulation taking place;

† indicates copulation which did not result in litter;

‡ indicates copulation resulting in a litter.

was known by functional tests with normal males to be fertile, and known by the vaginal smear and behavior tests to be in the pro-oestrous or early oestrous stage. The following morning careful search of the vaginal canal was made for the presence of the copulatory plug or of residual spermatozoa. The animals were as a rule tested in this way once monthly, but if for any reason the test was unsatisfactory (due, for instance, to the indisposition to copulate found in late stages of beri beri) repeated

tests were made thrice weekly.[6] The results of these tests are given in Table I, and graphs 1, 2, 3, and 4 show the composite and individual growth curves respectively. It will be seen that on the inadequate levels of yeast regardless of whether 200 milligrams or 100 milligrams were fed,



GRAPH 3. Individual curves of males reared on Diet 55+6 drops wheat germ oil+0.2 gm. yeast daily.
° indicates introduction of an oestrous female into cage of the male but without copulation taking place;
† indicates copulation which did not result in litter;
‡ indicates copulation resulting in a litter.

the animals were badly stunted in their growth. Since their life was prolonged for some ten months before death from beri beri intervened,

[6] As regards sex behavior, while our data shows serious disturbances in sex interest when animals are appreciably near the exitus lethalis, it is surprising how late normal sex interest and be-

TABLE I.

Diet 55: Casein (extracted) 18, cornstarch (cooked) 54, lard 22, cod liver oil 2, salts 4.

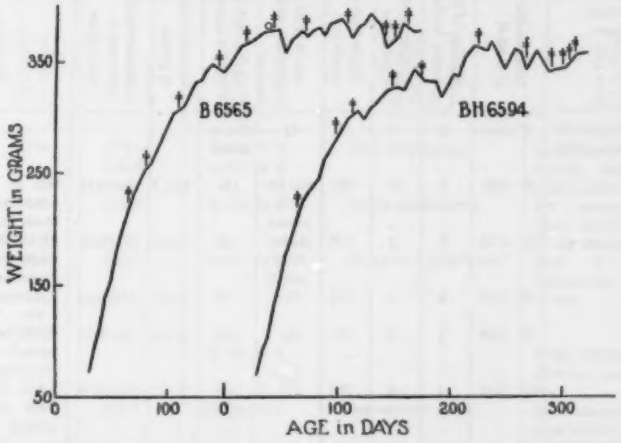
SHOWING THE RETENTION OF NORMALITY OF THE TESTES OF ANIMALS REARED WITH INADEQUATE LEVELS OF THE ANTINEURITIC VITAMIN (ADEQUATE LEVEL OF VITAMIN E BEING PRESENT) GROWTH CURVES OF THESE ANIMALS ARE DEPICTED IN FIGURES 1, 2, 3, 4.

Nutritional Regimen	Designation of animal	Number of instances in which an oestrous female was introduced into male's cage during experiment	Number of instances in which copulation occurred	Age in days at last copulation	Occurrence of beri beri	Age in days at autopsy	Sex activity as determined by observations	Autopsy Findings		
								Weight of testis (in grams)	Measurement of testes (in millimeters)	Histology of testes
Diet, 55, plus 0.8 dried yeast plus 6 drops wheat germ oil daily	BH 6565	13	11	326	—	334	Normal at 333 days	R: 1.53	R: 23x11x11.5	Normal
	W 6622	8	5	178	—	199 (died)	Not studied			
	B 6594	14	14	326	—	334	Normal at 333 days	R: 1.57	R: 22x13x12	Normal
Diet 55, plus 0.2 gram dried yeast plus 6 drops wheat germ oil daily	BH 6566	9	6	276	—	303 (dying)	Normal at 276 days		R: 22x12x12 L: 22x11x10	Normal
	B 6596	15	10	279	—	303	Normal at 279 days	R: 1.25		Normal
	B 6624	7	5	229	—	241 (died)	Not studied			
Diet 55, plus 0.1 gram dried yeast plus 6 drops wheat germ oil	W 6623	9	7	257	+	270 (died)	Not studied			
	W 6612	19	10*	242	+	270	Not studied	L: 1.59	L: 17x 8x10 R: 20x10x 9	Normal
	W 6619	12	8	244	+	270 (died)	Not studied			

* Seven of these matings resulted in failure of implantation in the females; first litter sired at 214 days.

abundant opportunity was given to test sex behavior and the fate of over forty copulations. The sexual physiology of the badly dwarfed males was practically normal up to within a few days of actual death.

In the case of the experiments conducted with Diet 228 the animals



GRAPH 4. Individual curves of males reared on Diet 55+6 drops wheat germs oil+0.8 gm. yeast.

° indicates introduction of an oestrous female into cage of the male but without copulation taking place;

† indicates copulation which did not result in litter;

‡ indicates copulation resluting in a litter.

were arranged in groups of six. Only two levels of yeast were employed, an adequate level (0.5–0.7 gram daily) and an inadequate one (0.1 gram daily). The *groups were of littermate brother controls with wheat germ oil and without wheat germ oil*, but otherwise the experiment was in every way conducted as was the preceding one, save that matings were attempted twice monthly instead of at rarer intervals. In every respect the same precautions were taken, i.e., only females known to be in active oestrus were employed. In the case of this group, still more frequent matings were

havior may be retained. In the case of animals BH 6566 and W 6596 on 0.2 gram yeast the tests were made three days apart. The two rats were tested with oestrous females at time indicated on their individual growth curves as shown in graph 4. It so happens that the first hourly test displayed no sex interest or activity but in each instance an identical test carried out three days later gave approximately normal sex activity, i.e., in the case of BH 6566 twenty-eight copulatory attempts and one plug formed during the hour and in the case of W 6596 twenty-seven copulatory attempts and one plug. *Both animals were dead three days after the tests!*

TABLE II

SHOWING THE DEPENDENCE OF TESTICULAR NORMALITY ON THE PRESENCE OF VITAMINE WHETHER ANIMALS ARE REARED ON INADEQUATE OR NORMAL LEVELS OF THE ANTINEURITIC VITAMIN. GROWTH CURVES OF THESE ANIMALS ARE DEPICTED IN GRAPHS 5, 6, 7, 8, 9.

Nutritional Regimen	Designation of animal	Number of litters sired during fertile period	Number of sterile copulations	Age in days at last copulation	Age in days at appearance of sterility	Removal of one Testis			
						Age in days at time of removal	Weight of testis in grams	Measurement of testis in millimeters	Histological findings
Diet 228, plus 0.1 gram dried yeast daily	W 5162	2	0	83	91	Not removed			
	W 5133	0	13	282	No fertility period	156	L-1.2	20x10x11	One or two tubules degenerated—others appear normal
	B 5126	0	2	139	No fertility period	146	L-1.0	20x10x10	All tubules show some degeneration Fused sperm
	W 5213	6	6	212	172	150	L-1.5	22x11x12	Appears normal
	W 5208	2	0	143	143	152	L-1.4	21x10x11	Some tubules appear normal—others show degeneration
	GH 5222	1	10	208	115	151	L-1.2	22x10x10	Some tubules filled with debris—others normal
Diet 228 plus 0.1 gram dried yeast, plus 6 drops wheat germ oil daily	W 5163	7	1	219	219	Not removed			
	W 5134	8	0	197	Never sterile	Not removed			
	W 5127	6	0	203	Never sterile	154	L-1.5	22x11x11	Normal
	W 5214	15	0	225	Never sterile	151	L-1.5	22x11x11	Normal
	G 5209	16	0	276	Never sterile	152	L-1.4	21x11x11	Normal
Diet 228 plus 0.5 –0.7 gram dried yeast daily	BH 5160	3	14	491	139	154	L-1.4	21x12x11	Normal
	W 5131	4	20	518	134	154	L 1.7	22x12x11	
	BH 5119	5	16	356	128	152	L-1.5	22x11x11	
	W 5206	3	4	179	117	151	L-1.1	20x1,1x11	Degeneration in most tubules
	W 5220	3	18	487	128	149	L-1.7	22x12x11	Degeneration in every tubule
	W 5212	3	17	452	129	228	L-1.0	18x 8x 8	Degeneration in every tubule
Diet 228, plus 0.5 –0.7 gram dried yeast, plus 6 drops wheat germ oil daily	BH 5161	10	9	678	343	154	L-1.3	21x12x10	Normal
	W 5132	8	5	524	345	Not removed			
	BH 5135	10	2	408	344	Not removed			
	W 5120	9	2	400	379	156	L-1.8	23x12x12	Normal
	W 5207	12	9	676	376	152	L-1.7	21x12x11	Normal
	W 5221	8	6	450	279	149		22x12x11	Normal

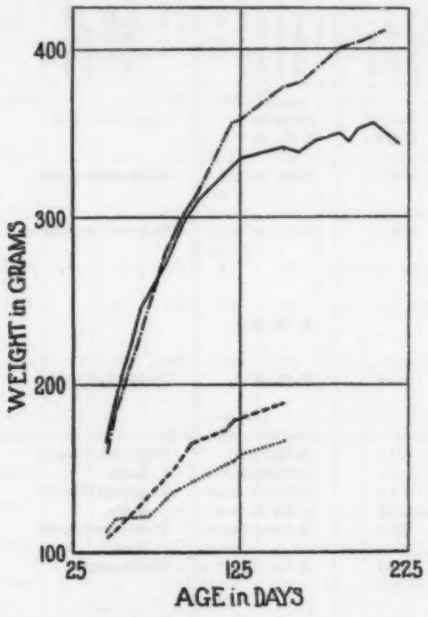
TABLE II (Continued)

SHOWING THE DEPENDENCE OF TESTICULAR NORMALITY ON THE PRESENCE OF VITAMIN E WHETHER ANIMALS ARE REARED ON INADEQUATE OR NORMAL LEVELS OF THE ANTINEURITIC VITAMIN GROWTH. CURVES OF THESE ANIMALS ARE DEPICTED IN GRAPHS 5, 6, 7, 8, 9.

Occurrence of beri beri	Age in days at autopsy	Autopsy Record			
		Weight of testis in grams	Measurement of testis in millimeters	Observation of fluid from epididymis	Histological findings
+	166	R-0.51 L-0.60	R-17x 8x 8 L-17x 8x 8	Non-motile sperm	Some degenerated tubules, fused sperm, a few normal tubules
+	312	R-0.5	R-16x 9x 8	Feebly motile sperm	No normal tubules about one-fourth completely degenerated
+	210	L-0.3	R-10x 7x 7	No sperm in epididymis	Mass of calcareous tubules—no normal tubules
+	239 Died				
No note	280		R- 9x 5x 5		Many calcareous tubules—no normal tubules
+	271	R-0.5	R-15x 9x 9	Degenerated sperm	All tubules partially degenetated—one-half completely degenerated
+	285	R-1.1 L-1.1	R-20x 9x10 L-20x10x10	Thousands of motile sperm	Normal
+	209	R-1.3 L-1.3	R-20x 8x 8 L-20x 8x 8	Thousands of motile sperm	Normal
+	210	R-1.6	R-22x11x11	Thousands of motile sperm	Normal
+	270	R-1.2	R-21x11x11	Motile sperm	One or two tubules look normal—others degenerated
+	287	R-1.6	R-22x12x12	Motile sperm	Normal
–	670 Died				
–	682 Died				
–	427	R 0.8	R-19x 9x 9	No sperm in epididymis	All tubules badly degenerated
–	189	R-0.5	R-17x 9x 9		Marked degeneration
–	651 Died				
–	685	R-0.9			
–	693	R-0.9	R-19x10x10		
–	685 Died				
	317 Died				
–	430	R-2.0	R-25x12x12	Millions of motile sperm	Normal
–	691	R-0.7	R-19x 8x 8		
–	689	R-0.7			

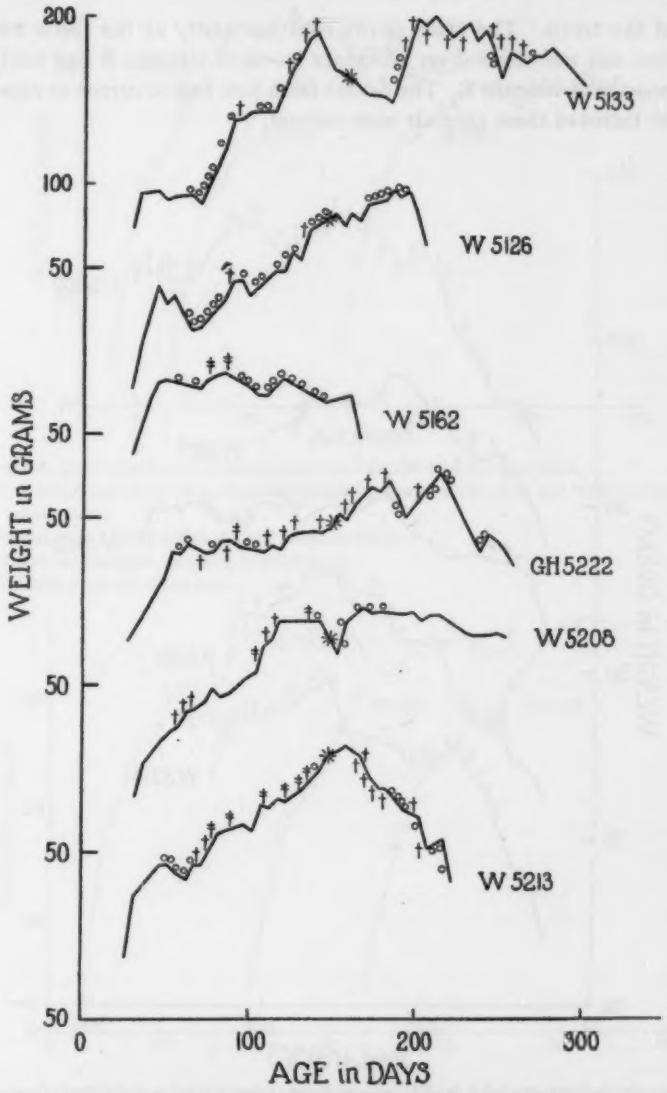
Note: In instances where animals died, no tissue was saved.

made late in the animal's life and at times which we know to have just preceded the outspoken and fatal symptoms of beri beri. Table II and graphs 5, 6, 7, 8, and 9 give the results of these experiments graphically. The graphs show strikingly the preservation of sex interest (as denoted by



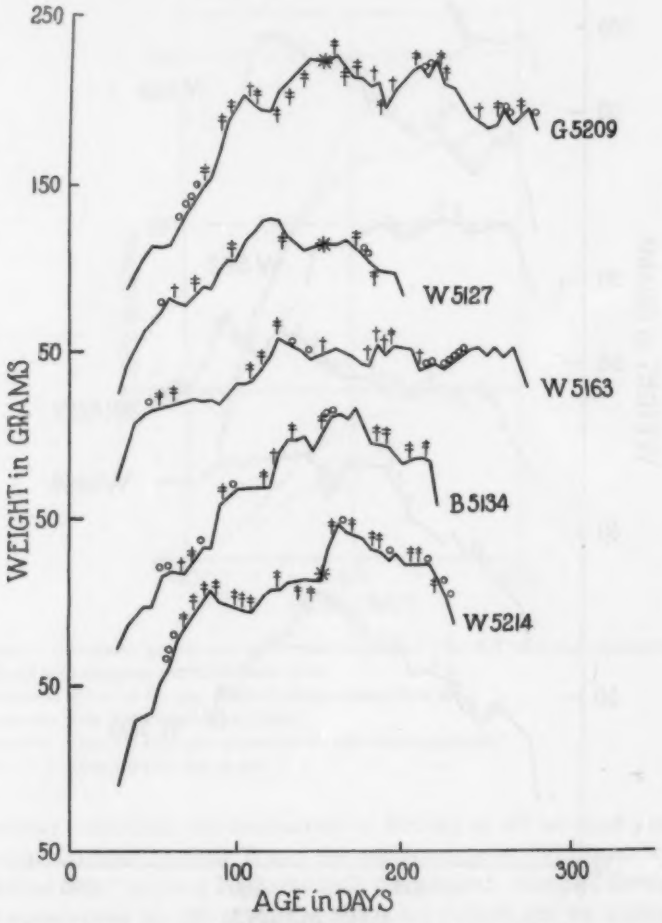
GRAPH 5. Composite growth curves of males reared on Diet 228 with and without wheat germ oil and with adequate and inadequate yeast.
—·—·—· 228+0.5-0.7 gm. yeast+6 drops wheat germ oil;
———— Diet 228+0.5-0.7 gm. yeast;
– – – – – Diet 228+0.1 gm. yeast and 6 drops wheat germ oil;
· · · · · Diet 228+0.1 gm. yeast.

copulations occurring) yet destruction of fertility at all levels of yeast in the groups without vitamin E and the preservation of fertility as well as sex interest at all levels of B providing E was present. Graph 7 shows that fertile copulations by the stunted males were carried out by animals so defective as to be constantly declining in weight. Table 2 includes the autopsy findings. When fluid is instantly expressed from the epididymis to a slide on the warm stage, the motility of spermatozoa is easily tested. The histological note was always made from a complete transverse sec-



GRAPH 6. Individual curves of males reared on Diet 228+0.1 gm. yeast
° indicates introduction of an oestrous female into cage of the male but without copulation taking place;
† indicates copulation which did not result in litter;
‡ indicates copulation resulting in a litter.
• indicates removal of left testis.

tion of the testis. The table shows that normality of the testis and its secretion did not depend on adequate levels of vitamin B but solely on the presence of vitamin E. The deaths from beri beri occurred as expected but the testes of these animals were normal.



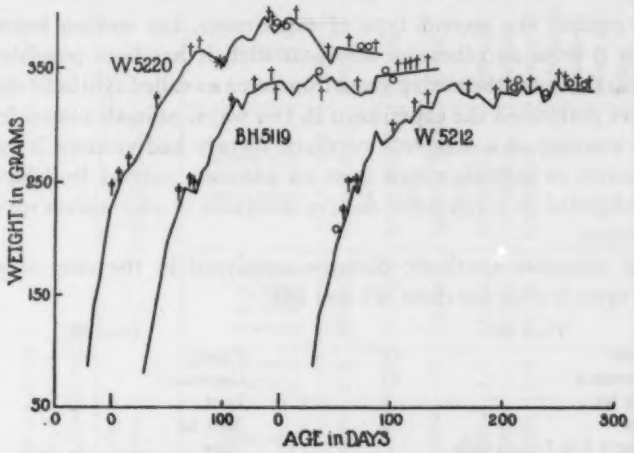
GRAPH 7. Individual curves of males reared on Diet 228+0.1 gm. yeast+6 drops wheat germ oil.

° indicates introduction of an oestrous female into cage of the male but without copulation taking place;

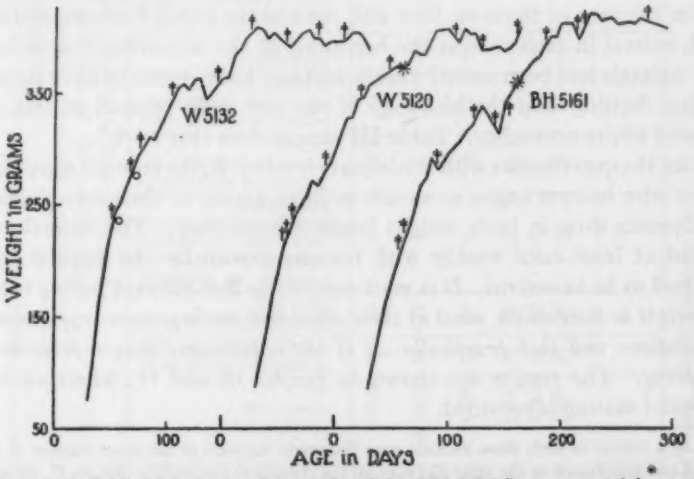
† indicates copulation which did not result in litter;

‡ indicates copulation resulting in a litter;

• indicates removal of left testis.



GRAPH 8. Individual curves of males reared on Diet 228+0.5–0.7 gm. yeast.
° indicates introduction of an oestrous female into cage of the male but without copulation taking place;
† indicates copulation which did not result in litter;
‡ indicates copulation resulting in a litter;
* indicates removal of left testis.



GRAPH 9. Individual curves of males reared on Diet 228+0.5–0.7 gm. yeast+6 drops wheat germ oil.
° indicates introduction of an oestrous female into cage of the male but without copulation taking place;
† indicates copulation which did not result in litter;
‡ indicates copulation resulting in a litter;
* indicates removal of left testis.

As regards the second type of experiment, the sudden lowering of vitamin B from an otherwise adequate diet, it has been possible to accomplish this by withdrawing yeast from some so-called synthetic dietaries. We have performed the experiment in two ways, animals reared from the day of weaning on a complete synthetic dietary had vitamin B suddenly substracted, or animals reared upon an adequate natural food have then been subjected to a synthetic dietary adequate in all respects save for B component.

The adequate synthetic dietaries employed in the case of animals reared upon it were our diets 201 and 202.[7]

Diet 201		Diet 202	
Casein	18	Casein	18
Cornstarch	73	Cornstarch	63
Milk fat	5	Lard	10
Salts	4	Milk fat	5
Yeast 0.5–0.7 gram daily		Salts	4
Wheat germ oil 6 drops daily		Yeast 0.5–0.7 gram daily	
		Wheat germ oil 6 drops daily	

The experiment of withdrawing B from the three individuals receiving wheat germ oil was carried out at the beginning of the fifteenth month of life in the case of animals on Diet 201 and at the eighth month of life in the case of those on Diet 202. One of the testes was removed from each animal in each case at the beginning of the withdrawal experiment. The animals had been mated weekly and had been shown to have retained perfect fertility and the histology of the one testis secured at this time showed entire normality. Table III summarizes this work.

Like the experiments with inadequate levels of B, the animals were placed upon wire bottom cages so as not to have access to their own feces. A precipitous drop in body weight began immediately. The animals were mated at least once weekly with females known by the vaginal smear method to be in oestrus. *It is most remarkable that although falling rapidly in weight to their death, most of these attempted matings were represented by copulations and that practically all of the copulations were represented by offspring.* The results are shown in graphs 10 and 11, where each attempted mating is recorded.

[7] As a matter of fact, these animals were littermate brothers of the same number of males reared and maintained on the same diet save for the absence of the fertility vitamin E. All groups of these animals were bred weekly to fertile females and those not receiving wheat germ oil were individually proved sterile at times in the particular cases varying from four to thirteen months The males on Diet 202 were quite suddenly sterile between the fourth and fifth month, but those on Diet 201 lost their fertility at various times beginning at the fourth month in one case but not until the sixth, seventh, and, in two instances, the thirteenth month.

TABLE III.

SHOWING THE RETENTION OF NORMALITY OF THE TESTES OF ANIMALS ON A COMPLETE SIMPLIFIED DIET FROM WHICH THE ANTINEURITIC VITAMIN WAS SUBSEQUENTLY WITHDRAWN

GROWTH CURVES IN THESE ANIMALS ARE DEPICTED IN FIGURES 10 AND 11.

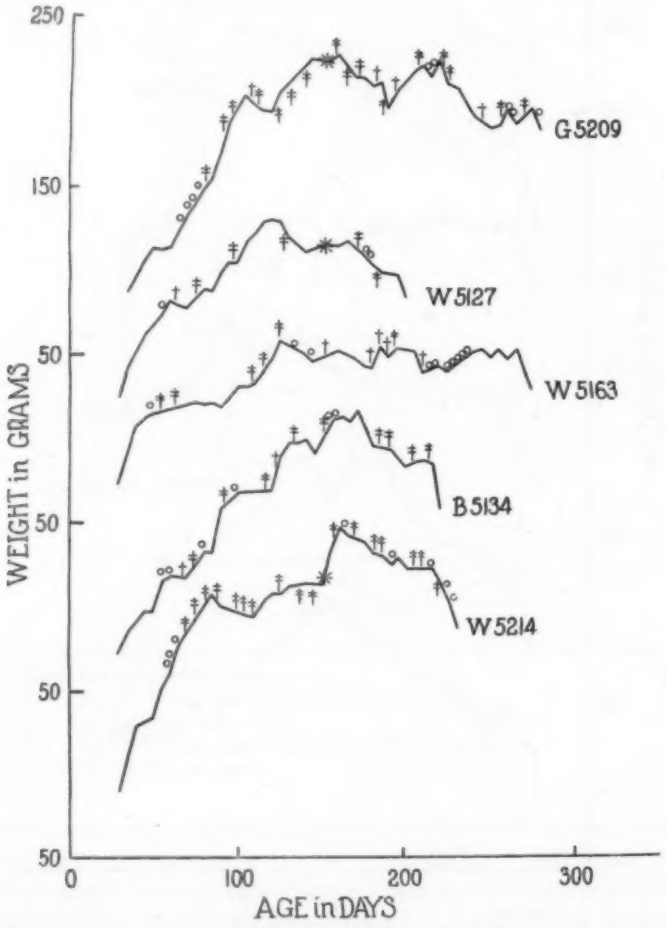
Diet 201—Casein 18, cornstarch (cooked) 73, milk fat 5, salts 4.

Diet 202—Casein 18, cronstarch (cooked) 63, milk fat 5, lard 10, salts 4.

Nutritional Regimen	Designation of animals	Number of litters sired before change of diet	Time in days on deficient diet	Number of instances in which an oestrus female was introduced into male's cage during experiment	Number of instances in which copulation occurred	Length of time in days from last copulation to autopsy	Occurrence of beri beri	L. testis removed the day yeast was withdrawn from diet			AUTOPSY RECORD			
								Weight of testes in grams	Measurement of testes in millimeters	Histology	Weight of testis in grams	Measurement of testis in millimeters	Observation of fluid from epididymis	Histology
Diet 201, plus 6 drops wheat germ oil. (Yeast withdrawn at 15 mos. of age)	B 1230	16	26	4	1	20	+		25x12x10*	Normal	1.6	22x11x11	Sperm actively motile	Normal
	BH 1243	14	23	4	1	17	+	1.5	22x12x11	Normal	1.5	21x11x11	Sperm actively motile	Normal
	B 1249	16	26	4	2	12	+	1.8	23x10x12	Normal	1.5	22x11x11	Sperm actively motile	Normal
Diet 202, plus 6 drops wheat germ oil. (Yeast withdrawn at 8 mos. of age)	B 1228	18	36	17	12	7	+		28x13x11	Normal	1.5	22x10x11	Not studied	Normal
	B 1241	11	40	14	7	16	+		23x12x10	Normal	1.1	18x11x 9	Not studied	Normal
	B 1247	14	50	15	4	37	+		26x13x12	Normal	0.8	18x 9x 9	Not studied	A very few tubules degenerated, others normal

* Testis removed 7 months before change of diet.

tion of the testis. The table shows that normality of the testis and its secretion did not depend on adequate levels of vitamin B but solely on the presence of vitamin E. The deaths from beri beri occurred as expected but the testes of these animals were normal.



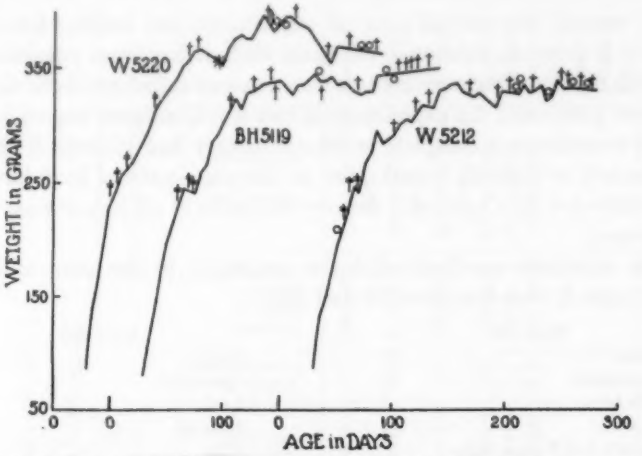
GRAPH 7. Individual curves of males reared on Diet 228+0.1 gm. yeast+6 drops wheat germ oil.

° indicates introduction of an oestrous female into cage of the male but without copulation taking place;

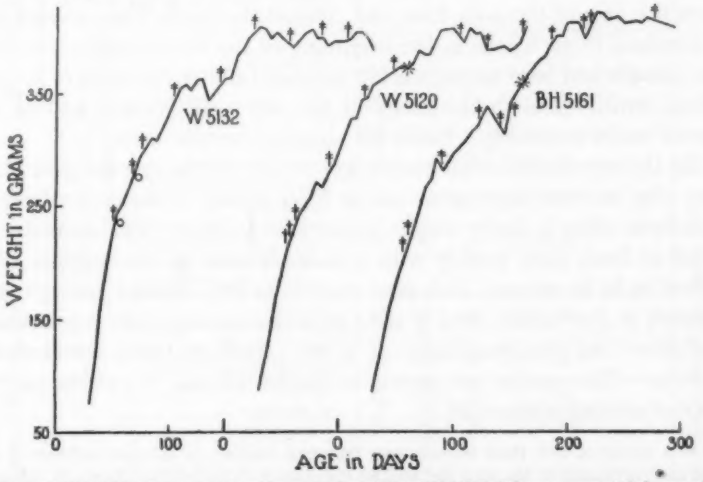
† indicates copulation which did not result in litter;

‡ indicates copulation resulting in a litter;

• indicates removal of left testis.



GRAPH 8. Individual curves of males reared on Diet 228+0.5–0.7 gm. yeast.
° indicates introduction of an oestrous female into cage of the male but without copulation taking place;
† indicates copulation which did not result in litter;
‡ indicates copulation resulting in a litter;
• indicates removal of left testis.



GRAPH 9. Individual curves of males reared on Diet 228+0.5–0.7 gm. yeast+6 drops wheat germ oil.
° indicates introduction of an oestrous female into cage of the male but without copulation taking place;
† indicates copulation which did not result in litter;
‡ indicates copulation resulting in a litter;
• indicates removal of left testis.

As regards the second type of experiment, the sudden lowering of vitamin B from an otherwise adequate diet, it has been possible to accomplish this by withdrawing yeast from some so-called synthetic dietaries. We have performed the experiment in two ways, animals reared from the day of weaning on a complete synthetic dietary had vitamin B suddenly substracted, or animals reared upon an adequate natural food have then been subjected to a synthetic dietary adequate in all respects save for B component.

The adequate synthetic dietaries employed in the case of animals reared upon it were our diets 201 and 202.[7]

Diet 201		Diet 202	
Casein	18	Casein	18
Cornstarch	73	Cornstarch	63
Milk fat	5	Lard	10
Salts	4	Milk fat	5
Yeast 0.5–0.7 gram daily		Salts	4
Wheat germ oil 6 drops daily		Yeast 0.5–0.7 gram daily	
		Wheat germ oil 6 drops daily	

The experiment of withdrawing B from the three individuals receiving wheat germ oil was carried out at the beginning of the fifteenth month of life in the case of animals on Diet 201 and at the eighth month of life in the case of those on Diet 202. One of the testes was removed from each animal in each case at the beginning of the withdrawal experiment. The animals had been mated weekly and had been shown to have retained perfect fertility and the histology of the one testis secured at this time showed entire normality. Table III summarizes this work.

Like the experiments with inadequate levels of B, the animals were placed upon wire bottom cages so as not to have access to their own feces. A precipitous drop in body weight began immediately. The animals were mated at least once weekly with females known by the vaginal smear method to be in oestrus. *It is most remarkable that although falling rapidly in weight to their death, most of these attempted matings were represented by copulations and that practically all of the copulations were represented by offspring.* The results are shown in graphs 10 and 11, where each attempted mating is recorded.

[7] As a matter of fact, these animals were littermate brothers of the same number of males reared and maintained on the same diet save for the absence of the fertility vitamin E. All groups of these animals were bred weekly to fertile females and those not receiving wheat germ oil were individually proved sterile at times in the particular cases varying from four to thirteen months The males on Diet 202 were quite suddenly sterile between the fourth and fifth month, but those on Diet 201 lost their fertility at various times beginning at the fourth month in one case but not until the sixth, seventh, and, in two instances, the thirteenth month.

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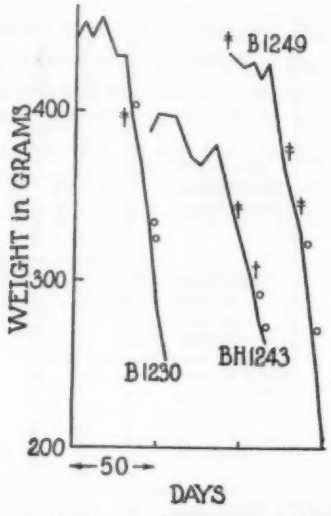
GROWTH CURVES IN THESE ANIMALS ARE DEPICTED IN FIGURES 10 AND 11.

Diet 201—Casein 18, cornstarch (cooked) 73, milk fat 5, salts 4.

Diet 202—Casein 18, cronstarch (cooked) 63, milk fat 5, lard 10, salts 4.

Nutritional Regimen	Designation of animals	Number of litters sired before change of diet	Time in days on deficient diet	Number of instances in which an oestrus female was introduced into male's cage during experiment	Number of instances in which copulation occurred	Length of time in days from last copulation to autopsy	Occurrence of beri beri	L. testis removed the day yeast was withdrawn from diet			Autopsy Record			
								Weight of testes in grams	Measurement of testes in millimeters	Histology	Weight of testis in grams	Measurement of testis in millimeters	Observation of fluid from epididymis	Histology
Diet 201, plus 6 drops wheat germ oil. (Yeast withdrawn at 15 mos. of age)	B 1230	16	26	4	1	20	+		25x12x10*	Normal	1.6	22x11x11	Sperm actively motile	Normal
	BH 1243	14	23	4	1	17	+	1.5	22x12x11	Normal	1.5	21x11x11	Sperm actively motile	Normal
	B 1249	16	26	4	2	12	+	1.8	23x10x12	Normal	1.5	22x11x11	Sperm actively motile	Normal
Diet 202, plus 6 drops wheat germ oil. (Yeast withdrawn at 8 mos. of age)	B 1228	18	36	17	12	7	+		28x13x11	Normal	1.5	22x10x11	Not studied	Normal
	B 1241	11	40	14	7	16	+		23x12x10	Normal	1.1	18x11x 9	Not studied	Normal
	B 1247	14	50	15	4	37	+		26x13x12	Normal	0.8	18x 9x 9	Not studied	A very few tubules degenerated, others normal

* Testis removed 7 months before change of diet.



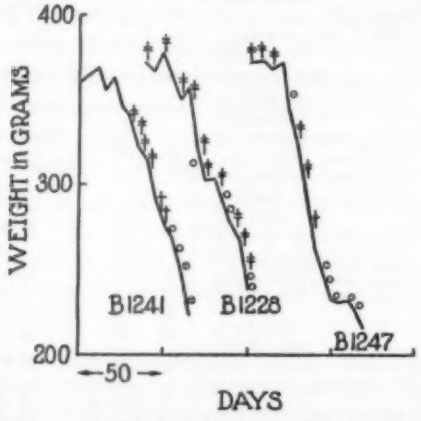
GRAPH 10. Individual curves of male rats showing the rapid decline when the yeast was withdrawn from the simplified diet on which they had been reared (Diet 201+6 drops wheat germ oil).

° indicates introduction of an oestrous female into cage of the male but without copulation taking place;

† indicates copulation which did not result in litter;

‡ indicates copulation resulting in a litter.

(Curves illustrate the period during which animal was on the diet deficient in Vitamin B)



GRAPH 11. Individual curves of male rats showing the rapid decline when the yeast was withdrawn from the simplified diet on which they had been reared (Diet 202+6 drops of wheat germ oil).

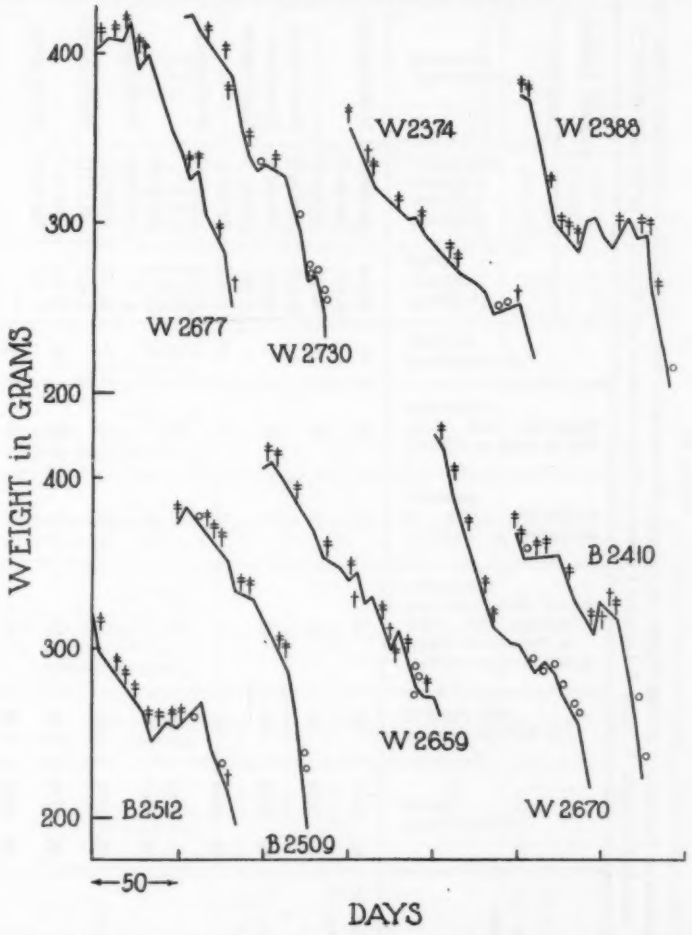
° indicates introuctiodn of an oestrous female into cage of the male but without copulation taking place;

† indicates copulation which did not result in litter;

‡ indicates copulation resulting in a litter.

(Curves illustrate the period during which animal was on the diet deficient in Vitamin B).

As has been said, a decline in weight came immediately and the animals quickly came to have a roughened fur and an enfeebled condition, with advancing emaciation. In all cases in the last week and in most cases in the last two weeks of their life, they exhibited to an outspoken extent



GRAPH 12. Individual curves of male rats showing rapid decline when taken from a natural food diet and put on a diet deficient in Vitamin B (Diet 263: Casein 20, cornstarch 55, milk fat 15, salts 5).

° indicates introduction of an oestrous female into cage of the male but without copulation taking place;

† indicates copulation which did not result in litter;

‡ indicates copulation resulting in a litter.

TABLE IV.

SHOWING THE RETENTION OF NORMALITY BY THE TESTES OF ANIMALS REARED ON NATURAL FOODSAND TRANSFERRED TO SIMPLIFIED DIETS LOW IN THE ANTINEURITIC VITAMIN B GROWTH CURVES OF THESE ANIMALS ARE DEPICTED IN FIGURES 12 AND 13

Nutrition Regimen	Designation of animal	Number of days on deficient diet	Number of instances in which an oestrous female was introduced into male's cage during experiment	Number of instances in which copulation occurred	Length of time in day from last copulation to autopsy	Occurrence of beri beri	Autopsy Record			
							Weight of testis in grams	Measurement of testis in millimeters	Observation of fluid from	Epididymis Histology
Diet 263, Casein 20, cornstarch 55, milk fat 15, salts 5.	B 2410	89	11	8	18	+	R-1.3	20x10x10	Motile sperm.	Normal
							L-1.3	20x10x10		
	B 2509	86	11	8	15	+	R-1.2	21x10x10		
							L-1.3	21x11x10	Motile sperm.	Normal
	W 2670	108	12	5	53	+	R-0.9	19x10x 9	Normal non-	Normal
							L-1.0	19x10x 9	Motile sperm.	
	W 2659	127	17	12	12	+	R-1.5	22x12x11		
							L-1.5	22x12x11	Motile sperm.	Normal
	B 2512	98	12	9	8	+	**			
	W 2388	99	12	11	9	+	R-1.3	20x10x10		
							L-1.3	20x10x10	Motile sperm.	Normal
	W 2374	116	15	12	11	+	R-1.5	20x10x11		
							L-1.5	20x10x11	Motile sperm.	Normal
	W 2730	99	13	7	27	+	R-1.1	20x10x 9		
							L-1.1	20x10x10	Motile sperm.	Normal
	W 2677	98	10	9	6	+	**			

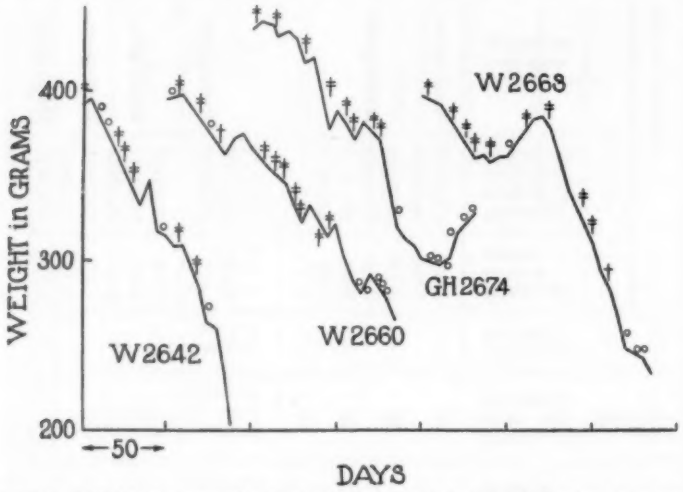
TABLE IV (*Continued*)

Nutritional Regimen	Designation of animals	Number of days on deficient diet	Number of instances in which an oestrous female was introduced into male's cage during experiment	Number of instances in which copulation occurred	Length of time in days from last copulation to autopsy	Occurrence of beri beri	Autopsy Record			
							Weight of testis in grams	Measurement of testis in millimeters	Observation of fluid from epididymis	Histology
Diet 264, Casein 20, cornstarch 55, milk fat 15, Vegex 0.5, salts 5.	W 2660	146	22	10	29	+	R-1.6 L-1.6	20x11x12 21x12x12	Motile sperm.	Normal
	GH 2674	146	18	11	29	+	R-1.6 L-1.6	21x12x10 21x12x10	Motile sperm.	Normal
	W 2663	146	16	10	23	+	R-0.6 L-0.8	17x 9x 8 19x 9x 9		R. testis—most tubules show degeneration L. testis—no mature sperm
	W 2642	100	8	6	20	+	R-1.0 L-1.1	20x10x10 20x10x10	Motile sperm.	Normal
Diet 265, Casein 20, cornstarch 55, milk fat 15, Vegex 1.5, salts 5.	GH 2139	146	8	7	1	−	R-1.6 L-1.7	22x11x12 22x12x12	Motile sperm.	Normal
	G 2432	146	7	4	16	−	R-1.7 L-1.7	22x11x11 22x11x11	Motile sperm.	Normal
	G 2420	146	11	9	1	−	R-1.5 L-1.8	21x11x11 22x11x12	Motile sperm.	Normal

* Transferred to wire-bottom cages 74 days after change of diet.

** Animal died—no tissue saved.

what has been called in the literature of experimental nutrition, beri beri in the rat, though complete paralysis of the posterior extremities did not occur. Only during the few days preceding death was sex interest destroyed, but the normality of the glands was maintained as shown by the



GRAPH 13. Individual curves of animals showing rapid decline when taken from a natural food diet and put on a diet deficient in Vitamin B (Diet 264: Casein 20, cornstarch 55, milk fat 15, salts 5, Vegex 0.5).

° indicates introduction of an oestrous female into cage of the male but without copulation taking place;

† indicates copulation which did not result in litter;

‡ indicates copulation resulting in a litter.

examination of fluid from the epididymis at the autopsy table, where motile sperm were found, and by the histological examination of the testis in complete cross sections.

The sixteen animals reared on our best natural food (Diet I) and transferred to simplified diets low in vitamin B_7 when eight months of age also showed at the time of their autopsy (and the animals were killed when death from beri-beri seemed imminent) normal motile spermatozoa in the fluid from the epididymis. The detailed histories of these groups is given in Table IV, and the growth curves and attempted matings are depicted in figures 12 and 13.

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[9] The diets (Diet 263, Diet 264, Diet 265) in these experiments were chosen in order to repeat the work of Parkes and Drummond (*Roy. Soc. Proc. B.*, vol. 98, 1925).

These studies would appear to leave no room for doubt that both acute and chronic deficiency in the antineuritic vitamin—deficiency leading to death—leaves the anatomical and functional integrity of the male germ cell unaffected. It has a definite effect on sex interest, decreasing this, but an effect which may not be shown until a few days preceding exitus. Providing vitamin E is adequately high, injury to the productive function in the male caused by inadequate B would appear to be due solely to this interference with behavior and to no extent to actual injury of the male germinal elements.

RELATION OF VITAMIN E TO GROWTH AND VIGOR[1]

HERBERT M. EVANS
(Department of Anatomy, University of California, Berkeley, Cal.)

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IT HAS become increasingly evident that the fat soluble vitamin E does not stand merely in the special relation which we know it occupies with the physiology of reproduction—the foetal placenta and the testis degenerating in its absence. Evidence has recently been adduced to show that the absence of this vitamin from the mother's milk entails a peculiar paralysis disease in the suckling young.[2] It is apparent therefore that the

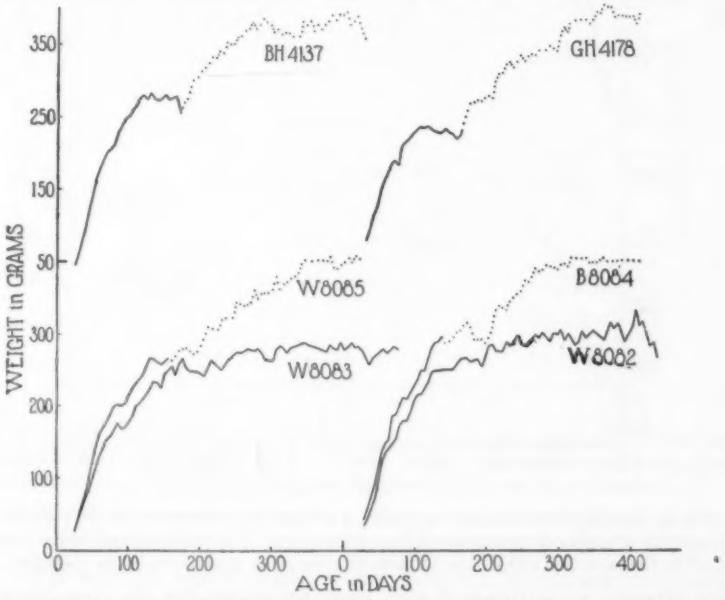

FIG. 1. Growth curve showing the marked improvement in growth of male rats when wheat germ oil (6 drops daily) was added to the basic diet. Fertility was not restored in these animals. The broken line indicates the period during which wheat germ oil was added to the diet.

[1] Aided by grants from the Committee for Research in Problems of Sex of the National Research Council and from the Bureau of Dairying of the United States Department of Agriculture. These funds have been generously augmented by the Board of Research and by the College of Agriculture of this institution.

[2] Evans, H. M. and Burr, G. O. Development of paralysis in the suckling young of mothers deprived of vitamin E, *Journ. Biol. Chem.*, 76, I, 273–297, Jan. 1928.

nervous system or, better, the neuro-muscular mechanism, is seriously injured if the vitamin is not conveyed to it at a critical period in the earliest phase of post-natal development.

We now desire to present evidence secured during the last two or three years showing uniformly improved growth and vigor in animals receiving

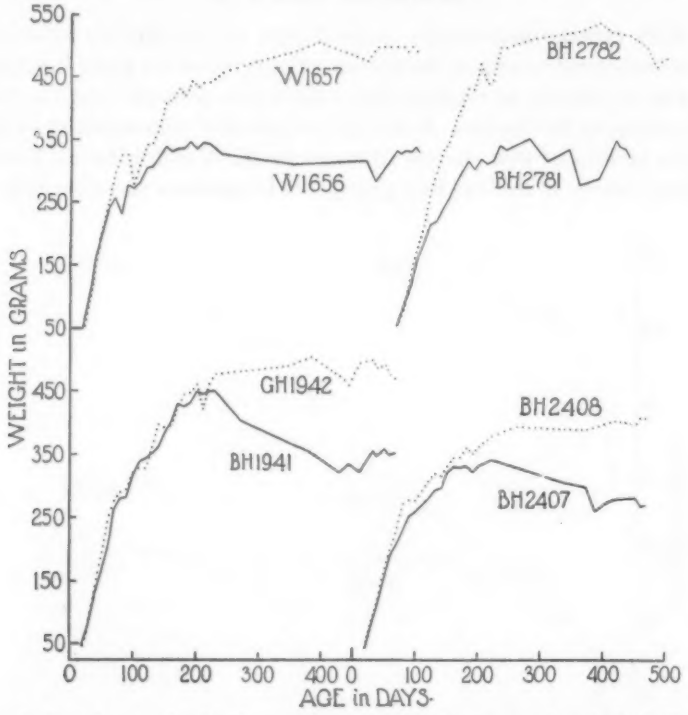

FIG. 2. Growth curve showing the superior growth in male rats reared on the basic diet with the addition of 6 drops of wheat germ oil daily (broken line). The growth of the littermate brothers reared on the basic diet without the addition of wheat germ oil is shown by the solid line.

this vitamin as contrasted with their littermate brothers or sisters on identical dietary regimes, save for the omission of E.

During our earlier efforts to cure male sterility due to low E, we encountered an increased growth in practically all animals receiving the few drops of wheat germ oil daily which we added for curative effect. In this early work we started the curative regime when sterility was well advanced, during the seventh or eighth month of the animal's life, or in some instances even later. In only about one-fourth of these cases were

we successful in the resuscitation of fertility.[3] In three-fourths of the cases sterility continued unabated, but in all of them the addition of the slight amount of wheat germ oil gave a clear response in improved growth and constitutional state of the animals so treated.

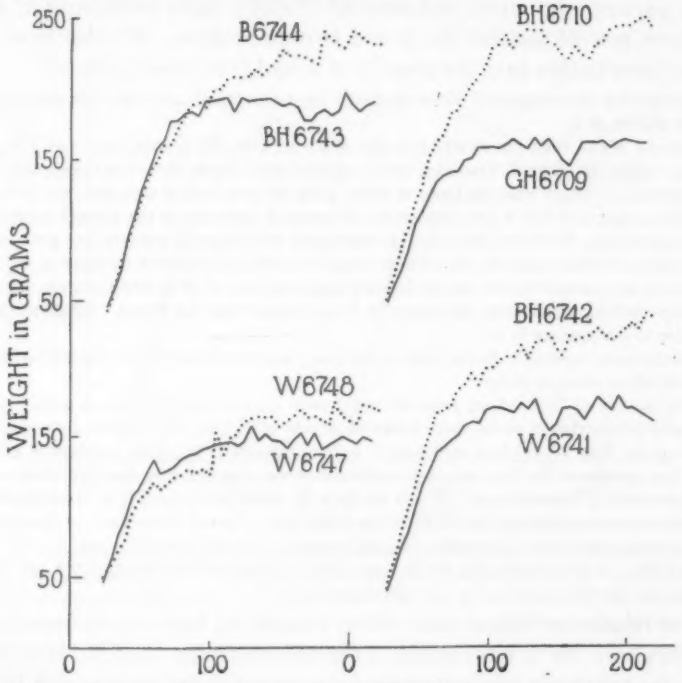

FIG. 3. Growth curve showing the superior growth in female rats reared on the basic diet with the addition of 6 drops of wheat germ oil daily (broken line) when compared with their littermate sisters reared on the basic diet without the addition of wheat germ (solid line).

A similar phenomenon is found in rearing animals on simplified dietary regimes, half of them, however, receiving a few drops of wheat germ oil daily. The animals receiving the wheat germ oil are almost invariably considerably heavier than their littermate controls.

The improvement in growth is not a sex-linked affair. Females, as may be seen from figure 3, show it as well as males.

To what nutritive improvement was this favorable effect due? It is not readily conceivable that the few added drops of wheat germ oil conferred

[3] As we have shown, even in cases where the curative regime was instituted as soon as sterility was detected no larger proportion of cures resulted. Evans, H. M., and Burr, G. O. The Anti-Sterility Vitamin Fat Soluble E, *Memoirs of the University of California*, vol. 8, p. 40. University of California Press, Berkeley. 1927.

a benefit by the slight additional fat to a diet which already contained 24% of fat. The improvement would therefore seem clearly due to a vitamin influence of this material. The possibility that it increased the resources in A or D would seem extremely remote, for 2% of the diet was constituted by a particularly potent cod liver oil (Patch), three milligrams of which we have proved satisfies the A and D requirements. We also have some direct information as to the paucity of A and D in wheat germ oil.

The level of wheat germ oil which markedly improves growth and vigor (six drops daily) is almost devoid of A.

Animals reared from the twenty-first day of life on Diet 251 (casein, extracted, 25%, cornstarch, cooked, 69, salts 4, irradiated lard 2) with 0.5 to 0.7 gram whole dried yeast daily do not live appreciably longer when six drops of wheat germ oil are added to their diet, nor is the characteristic sympton of low A (the appearance of cornified cells only in the vaginal smear) slower in its appearance. We have conducted an experiment with littermate sisters, one group without the addition of wheat germ oil, one with six drops of the oil, and one with six drops of the aerated (A-free) oil and, as said before, except for some postponement of 20 to 40 days in the appearance of xerophthalmia, substantial differences in these groups were not found. All of the animals died due to delinquency in A.

Furthermore, animals suffering from A deficiency are not relived by the administration of 18 drops of wheat germ oil daily.

The paucity of D in wheat germ oil would seem demonstrated by our experiments in the successful production in about three weeks of rickets with Diet 345 (yellow corn, ground 76, wheat gluten flour 20, calcium carbonate 3, sodium chloride 1) in all six members of a litter of rats. One member of the litter was then continued on the same diet save that the yellow corn was irradiated with a Hanovia lamp. Within ten days the initiation of healing as determined by the line test was quite evident at the proximal tip of the tibia. Four of the animals on the other hand were given in addition to their ration varying amounts of wheat germ oil, to wit: 1, 5, 20, and 60 drops daily. In no instance did the line test show healing had been inaugurated and it would appear that the wheat germ oil is virtually devoid of D.

The relation of wheat germ oil to vitamin A, however, is important in another way, for it has recently been demonstrated (Mattill, McCollum)[4] that the substance protects against the catalytic destruction of A induced by ferrous sulphate. We have hence to question whether wheat germ oil could have in this way protected the A content of our ration, the superior growth therefore resulting from improved A—a suggestion made by Mattill. All possibility that this is the case, however, is precluded by our manner of administration of the wheat germ oil which was not allowed to come in contact with the main ration (containing the A as 2% of cod liver oil) but was fed separately daily on the dose of yeast. Finally, that it is to the vitamin E content of the wheat germ oil that we must ascribe the improved growth and well-being would seem rather conclusively settled

[4] Mattill, H. A. The Oxidative Destruction of Vitamins A and E, *J. A. M. A.*, 89: (October 29) 1927; and

Simmonds, Nina, Becker, J. Ernestine, and McCollum, E. V., The Relation of Vitamin E to Iron Assimilation, *J. A. M. A.*, 88: 1047 (April 2) 1927.

by the attainment of this result when a highly concentrated sterol-free fraction of the non-saponifiable part of wheat germ oil is fed males.

The improvement in growth due to vitamin E characterizes only the last phase of growth in these animals, the phase intervening after the

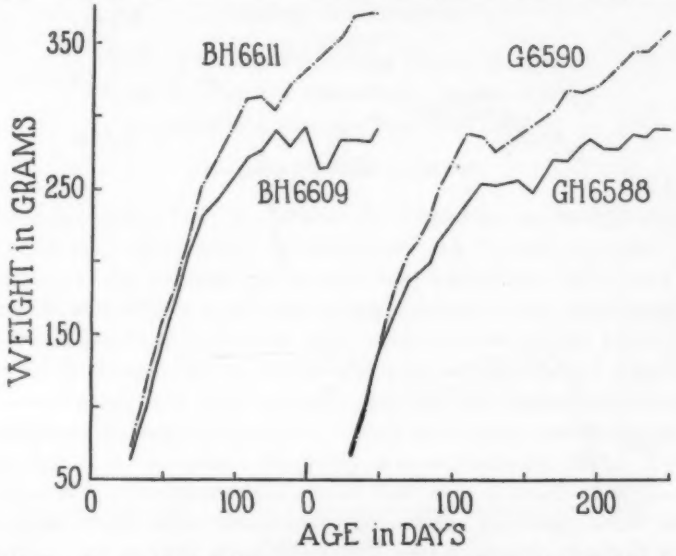

FIG. 4. Growth curve showing the superior growth of male rats reared on the basic diet with the addition of minute doses of a highly concentrated sterol-free fraction of the nonsaponifiable part of wheat germ oil—indicating, we believe, that improved growth is due to the presence of vitamin E and not to some other possible factor in the wheat germ oil. The growth of littermate brothers reared on the basic diet without the addition of this vitamin E concentrate is shown by the solid line.

attainment of maturity, which is usually viewed as a plateau in the growth curve though all animals show at this time a slow ascent. The improvement becomes increasingly evident after the eighth month of life, and is very marked after one year. It could therefore have been overlooked by investigators studying merely the completion of the energetic early growth. We have in fact recently shown that during the first ninety days of life of the female rat, significant differences may not occur in the growth on E high (natural food) and on E free (simplified) diets.

We next sought to inquire as to whether the improved growth could be ascribed to a favorable influence upon the testis. This organ, as is well known, intervenes in some way in the metabolism so as to give an increased growth and stature to the male and anything favoring it might have the same result. We could explain the resumption of growth in our

unsuccessful attempted cures of sterility with wheat germ oil, by postulating a favorable influence on the testis, even though the production of ripe germ cells was not brought about. A crucial attack upon this problem seemed to us only possible by the administration of the E rich oil after

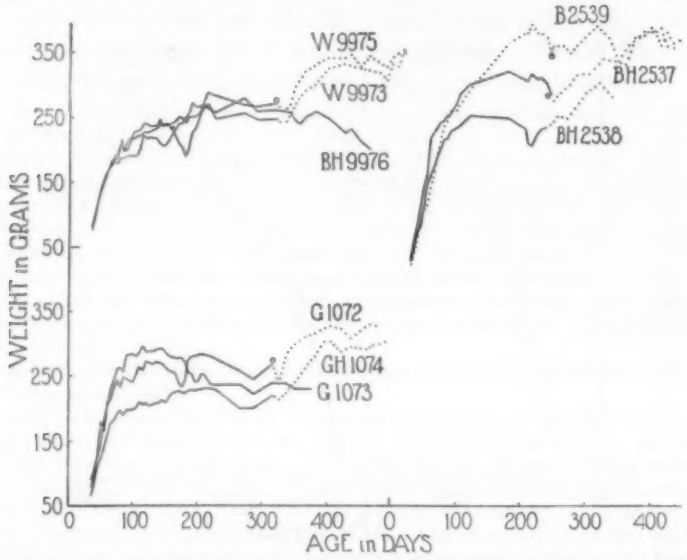

FIG. 5. Growth curves of male rats reared on the basic diet with and without the addition of vitamin E (in the form of wheat germ oil). In some individuals both testes were removed (0) and wheat germ oil added to the diet of the animals which had not previously received it. The broken line indicates the period during which wheat germ oil was added to the ration. Littermate brothers are grouped together.

the elimination of the sex glands. The usual superior growth was again evoked in these experiments.

It is apparent therefore that vitamin E does not exert its favorable effects upon growth and the general state of the animal indirectly through its established value to the sex glands, but in some other, possibly more direct way. The diminution in sex activity of males held upon E free regimes—a diminution shown only in the later stages of their lives—may not be due, it must be emphasized, to impairment of the testis,[5] *but may result from the constitutional inferiority of such animals— the special topic of the present paper.*

[5] We must of course bear in mind the possibility that E favors the intertubular testicular tissue, the tissue responsible for the retention of secondary sex characters and normal sex behavior in cryptorchism. A retention of normal sex responses occurs long after complete loss of the seminiferous epithelium produced by artificial cryptorchism.

SUPPLEMENTARY VALUES AMONG FOODS

II. GROWTH AND REPRODUCTION ON WHITE BREAD WITH VARIOUS SUPPLEMENTS

By MARY SWARTZ ROSE *and* GRACE MACLEOD
(From the Nutrition Laboratory, Teachers College, Columbia University, New York City)

Received for Publication—May 29, 1928

IN a former paper from this laboratory[1] it has been shown that excellent growth and reproduction of albino rats can be secured on a diet in which 50% of the calories are derived from white flour, 25% from dried whole milk and 10% from an assortment of common vegetables in proportions found practical in human experience, the rest of the ration being composed of dextrin, butter (or a substitute equally rich in vitamin A) and a small amount of meat residue. This diet has carried rats through 5 generations with improved growth in each succeeding generation in spite of the fact that the infant mortality was increasingly high. Cowgill[2] and some of his coworkers have shown that with as much as 80% of the total calories derived from cereal grains there can be good growth and reproduction, but in their diets the cereals were a mixture of whole grains, wheat, corn, and oats, the ash constituents were furnished in sufficient quantity in the form of a standard salt mixture, and cod liver oil insured adequate vitamin A. Furthermore, lettuce and liver or egg and molasses used to reinforce the diet so that any lack in the cereal was more than compensated by the excellent quality of the rest of the ration.

Sherman, Rouse, Allen and Wood[3] fed 28-day-old rats bread made with water and minimum amounts of salt, sugar, lard and yeast, but the animals immediately ceased to grow and died in about 6 weeks. When fresh apple was added to the diet in amounts equal to one-fourth of the total calories there was no growth, but the animals lived longer than on bread alone.

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[1] Rose and McCollum. Supplementary Values among Foods. I. Adding Egg to an Adequate Diet Compared with Increasing Vegetables. *Proc. Soc. Exper. Biol. Med.*, 25 (1928) 697–698.

[2] Cowgill, Jones, Frisch and Jackson. Studies on the Effects of Abundant Cereal Intake. I. The Use of Cereals as the Chief Source of Calories. *J. Am. Med. Assoc.*, 89 (1927) 1770–1774. Also II. The Use of Supplements Other than Milk. *J. Am. Med. Assoc.*, 89 (1927) 1930–1932.

[3] Sherman, Rouse, Allen and Wood. Growth and Reproduction upon Simplified Food Supply. *J. Biol. Chem.*, 46 (1921) 503.

Addition of meat resulted in growth for about five weeks, then rapid decline and death almost as soon as on bread alone. With equal weights of fresh bread and fluid milk there was normal growth through three generations.

Eddy[4] fed white bread made with and without milk and with varying amounts of yeast. In whole milk breads vitamin B appeared to be the first limiting factor. On commercial breads the animals began to decline in weight in less than 40 days and these breads required supplementing for proteins, ash constituents and vitamin A.

In the present experiments we have used rations in which 70% or more of the dry weight was furnished by bread made from high grade patent flour. Since white bread is not always made by the same formula, we endeavored to get representative results by using three kinds of bread. One was a commercial product (C), containing some milk, as well as lard, sugar, salt and yeast; the other two were specially prepared and contained the same amounts of yeast, but one (A) was made with water and the other (B) with fluid milk.

Their composition is given below:

TABLE I.

COMPOSITION OF BREADS A AND B

Ingredients	-A-	-B-
	Weight lbs.	Weight lbs.
Flour	100.0	100.0
Water	62.0	——
Sugar	3.5	1.5
Salt	1.75	5.0
Yeast	5.0	5.0
Milk, fresh	——	64.0
Malt	——	1.5
Butter	——	3.0

These breads were analyzed in the laboratory for total nitrogen by the Kjeldahl method; for carbohydrates as glucose by Allihn's method (after acid hydrolysis) and for fat by extraction with ether. The results are shown in the following table:

[4] Eddy. The Place in the Diet of White Bread made with Milk. *J. Home Economics.* 16 (1924) 428–438.

TABLE II.

1. ANALYSIS OF AIR DRY SAMPLES

Bread	Water %	Protein %	Fat %	Carbohydrate (directly determined) %	Ash %	Cal. per gm.
A	4.73	14.09	0.75	78.34	2.07	3.76
B	2.20	14.15	3.13	65.20	1.96	3.46
C	3.27	14.24	2.44	66.50	2.17	3.45
		2. RECALCULATED AS FRESH BREAD				
A	29.88	10.37	0.55	57.66	1.52	
B	24.99	10.85	2.40	50.01	1.50	
C	35.96	9.43	1.62	44.02	1.44	

The experimental animals were taken from parents on Sherman's diet 13, and placed at the age of 28 days on the experimental rations. They were given only distilled water to drink. Weekly records of weight and food consumption were kept. Six series of experiments were conducted using as supplements whole milk at two different levels, egg, carrot, spinach, almond and lean beef. These diets are shown in Table III.

TABLE III.

EXPERIMENTAL DIETS

Proportions of Bread and Supplement

Series 1.	a.	Dried Bread	70%
		Whole Milk Powder	30%
	b.	Dried Bread	90%
		Whole Milk Powder	10%
Series 2.		Dried Bread	70%
		Whole Egg Powder	30%
Series 3.		Dried Bread	70%
		Dried Carrot	30%
Series 4.		Dried Bread	70%
		Almond Meal	30%
Series 5.	a.	Dried Bread	97%
		Dried Spinach	3%
	b.	Dried Bread	95%
		Dried Spinach	5%
	c.	Dried Bread	90%
		Dried Spinach	10%

When dried whole milk powder constituted 30% of the weight of the ration the animals grew well, and, with one exception, all the females bore several litters of young, but barring one litter none of these lived more than a few days. Here vitamin B was probably the first limiting factor, as shown by Eddy. When the milk was reduced to 10% of the ration,

Chart No1

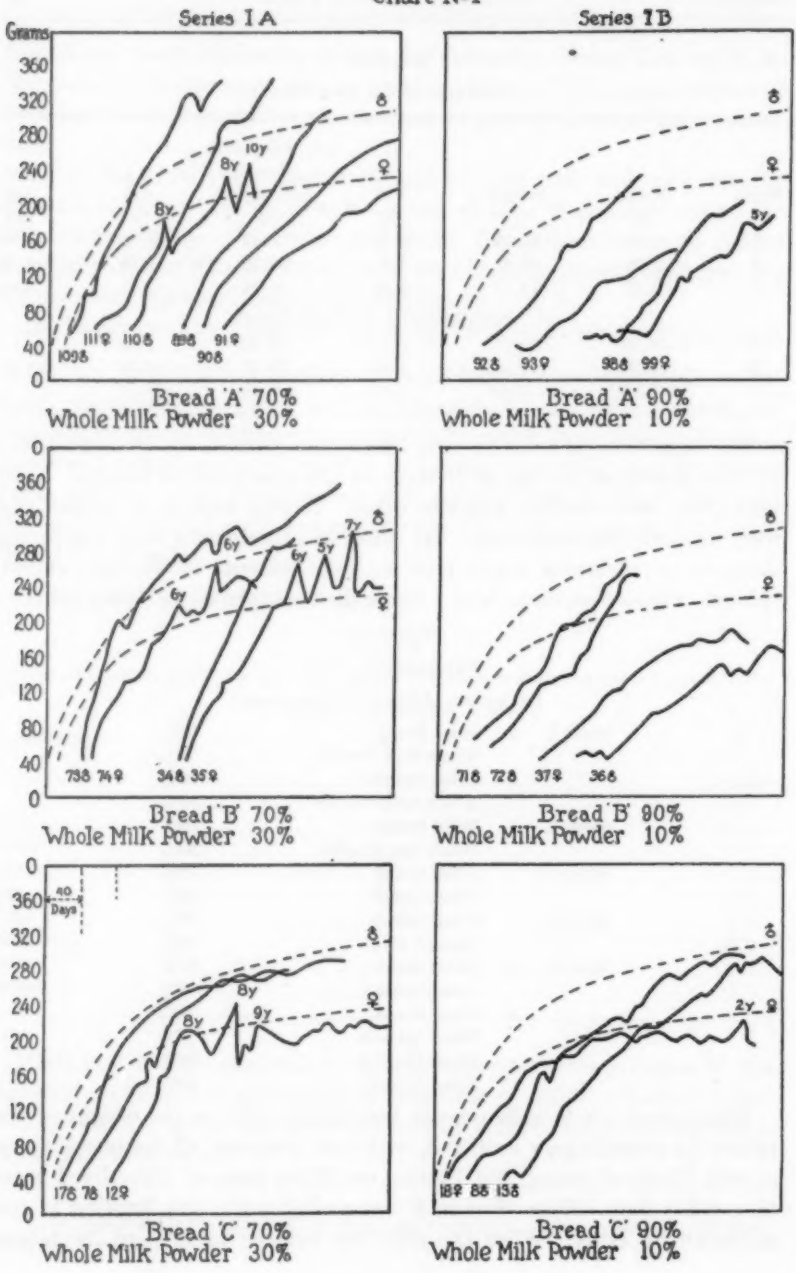
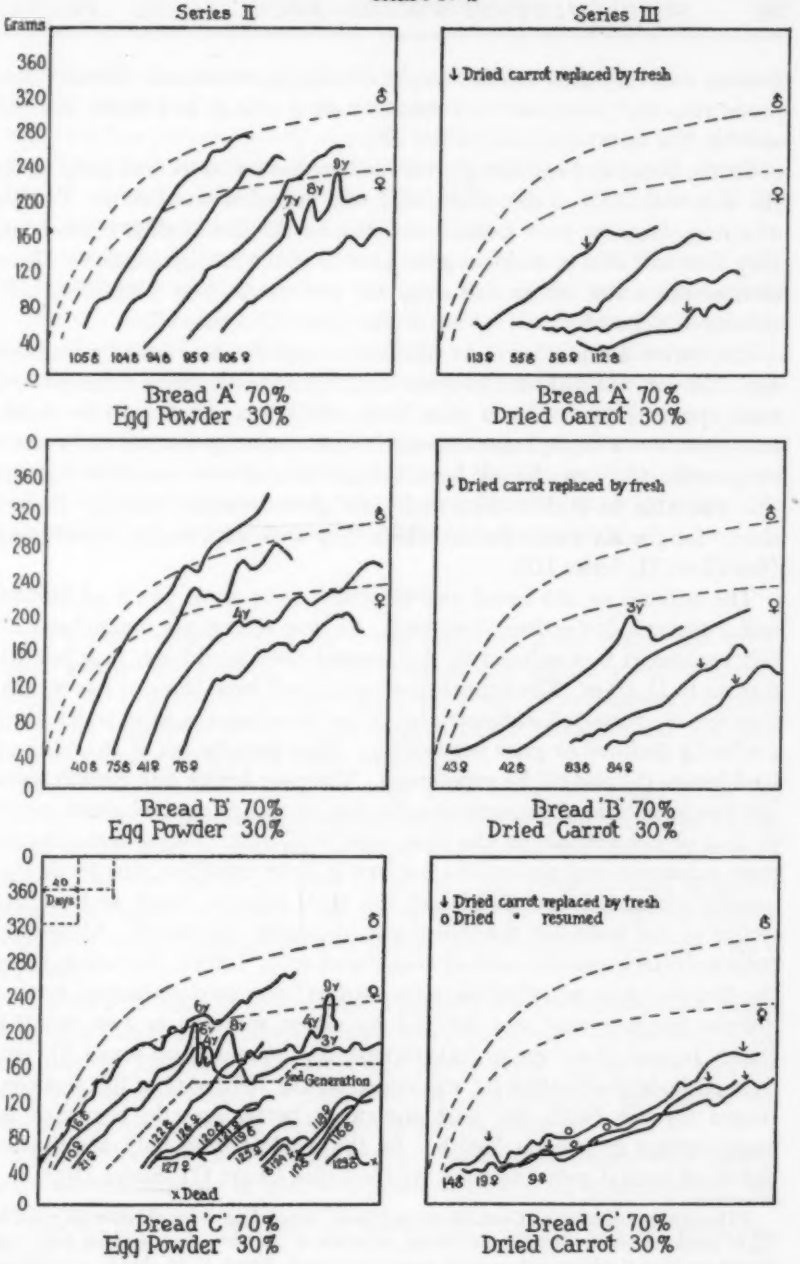

Series I A
Series I B
Grams
360
320
280
240
200
160
120
80
40
0
♂
♀
10y
8y
8y
111♀ 110♂ 88♂ 91♀
109♂ 90♂
92♂ 93♀ 98♂ 99♀
5y
Bread 'A' 70%
Whole Milk Powder 30%
Bread 'A' 90%
Whole Milk Powder 10%
6y
6y
6y
5y
7y
73♂ 74♀ 34♂ 35♀
71♂ 72♂ 37♀ 36♂
Bread 'B' 70%
Whole Milk Powder 30%
Bread 'B' 90%
Whole Milk Powder 10%
40 Days
8y
8y
9y
17♂ 78 12♀
18♀ 88 13♂
2y
Bread 'C' 70%
Whole Milk Powder 30%
Bread 'C' 90%
Whole Milk Powder 10%

Chart No2


Series II
Series III
Grams
360
320
280
240
200
160
120
80
40
0
♂
♀
9y
8y
7y
105♂ 104♀ 94♂ 95♀ 106♀
↓ Dried carrot replaced by fresh
113♀ 55♂ 58♀ 112♂
Bread 'A' 70%
Egg Powder 30%
Bread 'A' 70%
Dried Carrot 30%
4y
40♂ 75♂ 41♀ 76♀
43♀ 42♂ 83♂ 84♀
Bread 'B' 70%
Egg Powder 30%
Bread 'B' 70%
Dried Carrot 30%
40 Days
6y
5y
3y
2nd Generation
x Dead
↓ Dried carrot replaced by fresh
o Dried * resumed
148 19♀ 9♀
Bread 'C' 70%
Egg Powder 30%
Bread 'C' 70%
Dried Carrot 30%

growth was very slow at first, but gradually approximated normal. Reproduction was poor, only two females having young, and those did not survive (See Chart I, Series 1A and 1B).

On the bread and egg diet growth and reproduction were as good as on the diet with 30% of dry whole milk and lactation was better. Twelve offspring of one rat were weaned and kept on the diet of their mother but they were not able to make as good growth as the first generation. Three died within a few weeks and even the one which grew best (No. 122) developed rickets by the 17th week (See Chart II, Series II).

The carrot did not prove as efficient a supplement to bread as milk or egg. Growth was retarded in every case. The animals were lethargic and their appetites poor. For a time fresh carrot was substituted for dried, and there was a slight improvement in food consumption but little effect on growth. Only one female bore a single litter of very small young, but she was able to suckle them and they gained weight steadily though slowly for the six weeks during which they were kept under observation (See Chart II, Series III).

The animals on the bread and meat diet were given bread ad libitum and 5 grams a day of fresh lean beef. As they seldom ate more than half this amount it was reduced to the amount they would eat, in a few instances to 1½ gram. The animals grew quite well from three to five weeks, then usually remained stationary for two or three months, after which they gradually declined or grew very slowly. Five animals out of the 13 used died before the end of the experiment. The poor health and growth were not due to poor food consumption for that of this group was about equal to that of the animals on the diets with 30% milk. Nasal hemorrhages were numerous and severe, the fur was in poor condition, the teeth frequently elongated and discolored; the front legs too weak to bear the weight of the body and the thorax was frequently contracted. Altogether these animals presented marked evidence of acute rickets. At various times the diet was changed to include either butter fat or calcium lactate, but no definite improvement was secured by either measure in two months' time. In case of one group (rats number 54, 56 and 57 on bread B), cod liver oil instead of butter fat was added on the 209th day of life and continued till the 240th day with somewhat better growth, but with no improvement in general health. In the whole period only about one-eighth of normal gain had been attained (See Chart III, Series IV).[5]

[5] The changes indicated on Chart III are as follows: Bread A, addition of butter fat equal to 9% of weight of bread. Bread B, 1st change, reduction of meat from 5 to 2½ grams daily; 2nd change, addition of calcium lactate equal to weight of bread. Bread, C, 1st change, reduction of meat to 2½ grams daily; 2nd change, reduction of meat to 1½ grams daily; 3rd change, addition of cod liver oil equal to 2% of weight of bread.

Chart III also shows a series with bread 70% and dried whole almonds 30%. On this diet the food consumption was above average, but as reported in a previous study of almonds[6] the diet had a very bad effect on the fur, which looked as though it had been oiled. Animals surviving for six months attained only one-eighth of normal weight, and had bad nasal hemorrhages as well as much loss of hair.

In the first trials with dried powdered spinach shown in Chart IV 30% of the ration was derived from this food, but the animals either refused to eat or were overcome with gastro-intestinal disturbances and speedily died. The spinach was then tried at 3, 5 and 10% levels. The best results were obtained with the lowest percentage and even at this level the animals did not attain more than half normal size, although food consumption was up to the normal average. In the spinach, the concentration of ash constituents is very high, being 11.5% of the dry weight, as compared with 4.6% in the carrot and 2% in the almond. It is possible that something in the spinach is toxic when fed in large amounts.

Summary

On a diet composed of dried bread 70% and dried whole milk 30%, there was normal growth, excellent health, and good reproduction, only one of the five females failing to bear offspring. Four females produced 81 young within the period of the experiment but lactation was poor and only five young were successfully weaned.

Similar results were obtained with dried bread 70% and dried egg powder 30%. There was good reproduction, four females bearing 68 young, 12 of which were successfully weaned and placed on the diet of their mothers at the age of 28 days. Half of them made normal gains for periods of three to six months, but the other half all showed signs of general weakness, nasal hemorrhages and rickets, and three of these died within six weeks.

Results with bread 90% and milk 10% were similar to those with bread 70% and carrot 30%. Growth was poorer than with egg or 30% milk, and only one mother on the carrot diet bore young. These were successfully weaned and placed on the carrot diet, making good growth for the two months during which they were under observation.

Neither the bread and almond nor bread and spinach diets were able to maintain the animals in health in spite of good food consumption. At

[6] Rose and MacLeod. The Almond as a Source of the A Vitamin. *Proc. Soc. Exper. Biol. Med.*, 19 (1922) 391–393.

Chart No.3

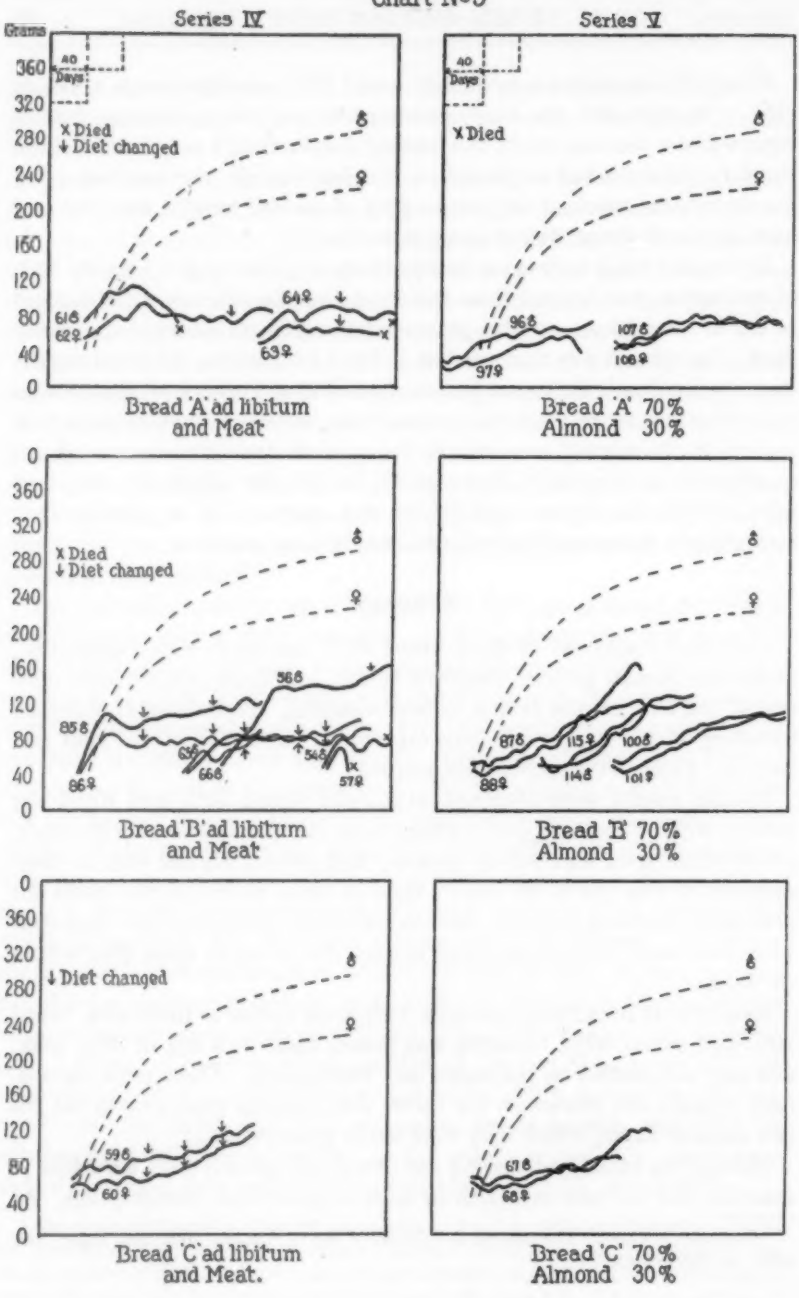
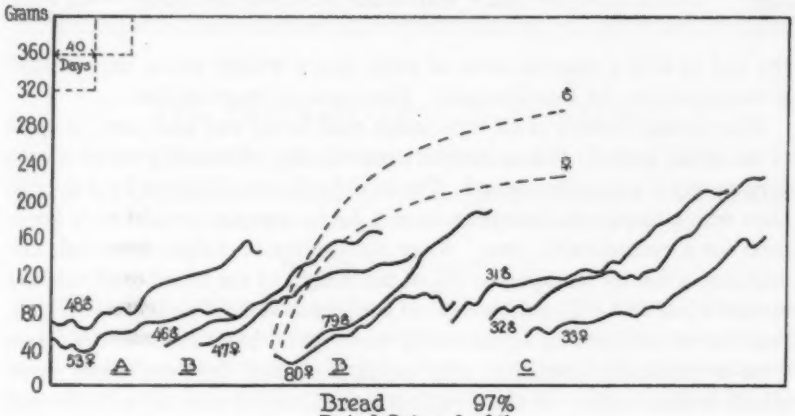
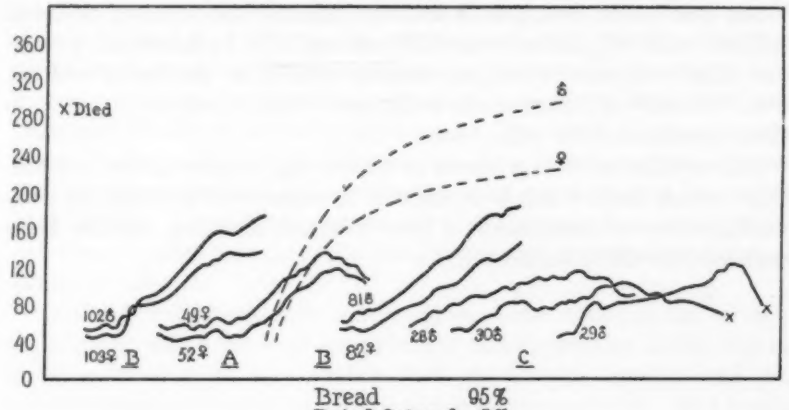

Series IV
Series V
Grams
40 Days
X Died
↓ Diet changed
♂
♀
616
622
64♀
63♀
96♂
97♀
107♂
106♀
Bread 'A' ad libitum and Meat
Bread 'A' 70% Almond 30%
85♂
86♀
56♂
66♂
54♂
57♀
87♂
88♀
115♀
114♂
100♂
101♀
Bread 'B' ad libitum and Meat
Bread 'B' 70% Almond 30%
59♂
60♀
61♂
68♀
Bread 'C' ad libitum and Meat.
Bread 'C' 70% Almond 30%

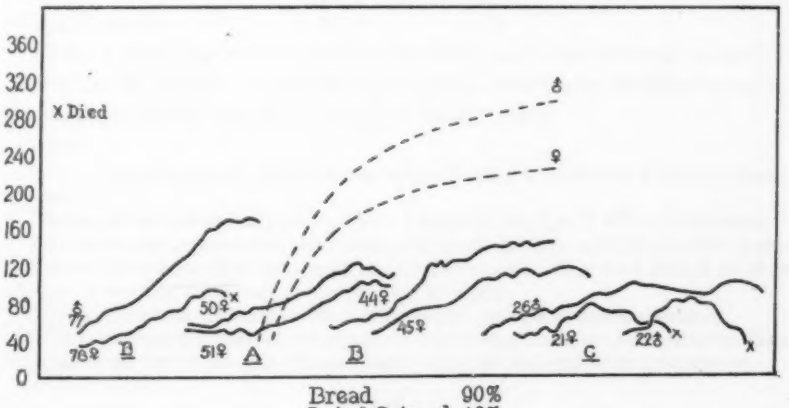
Chart No 4



Bread 97%
Dried Spinach 3%



Bread 95%
Dried Spinach 5%



Bread 90%
Dried Spinach 10%

the end of 6 or 8 months most of them had a weight which they should have reached in the second month. There was no reproduction.

The poorest records of all were made with bread and lean beef, in spite of an initial period of three to five weeks during which all grew at a rate very nearly if not quite normal. The initial gain was followed by a decline after which there was maintenance of a fairly constant weight on a lower level for a considerable time. After the decline in weight occurred, the addition of butter fat equal to 9% of the weight of the bread or of calcium lactate equal to 2.6% of the weight of the bread, had little effect on growth, but that of cod liver oil equal to 2% was slightly beneficial. The animals were in miserable condition, and so crippled that they could not move about in their cages. Of the 13 animals used, only 8 were alive at the end of eight months.

The best health, best growth and best reproduction occurred on bread 70% and milk 30% and on bread 70% and egg 30%. In the second generation there were more young successfully weaned on the bread and egg diet. No marked differences in result can be attributed to differences in the composition of the white bread.

Such studies serve as a means of discovering supplementary relationships among foods which it is essential to understand if we are to make intelligent use of assortments of food in human nutrition, and are being continued in this laboratory.

THE DISTRIBUTION OF VITAMIN E

NINA SIMMONDS, J. ERNESTINE BECKER *and* E. V. MCCOLLUM

(From the Biochemical Laboratory, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Md.)

Received for Publication—June 1, 1928

THE reported studies relating to sterility in rats brought about by diets deficient in vitamin E emphasize the richness of wheat germ oil and certain other vegetable products in this principle; its absence or paucity in lard and cod liver oil, and its relative scarcity in butter fat (1). Since we desired to produce female rats which would show resorption, and males which would suffer degeneration of germinal epithelium, we fed diets composed of yeast, casein, cod liver oil, salt mixtures, lard and dextrinized starch,[1] and were surprised to observe such high fertility as to compel the conclusion that those preparations of the fats which we used contained vitamin E in relative abundance.[2] That these fats served as the source of vitamin E must be deduced from the results of a number of control experiments carried out to test this point. A feature of the studies here reported was a further examination of the hypothesis which we at one time recorded that there is a relation between vitamin E and iron assimilation (2).

Tables I to III present in condensed form the results of the series of experiments which led us to conclude that certain samples of lard, instead of being free from E, may contain it in considerable amount, and that certain samples of cod liver oil are also good sources of it. Our results also indicate that 10% of butter fat as a source of E is inferior to 2, 3 or 4% of cod liver oil.

Table I gives the sources of E in the diets, and data relating to fertility or sterility in females. Table II supplies data relating to sterility in males. Table III contains complete formulas for the diets.

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[1] The yeast employed was a high grade powder furnished by the Northwestern Yeast Company, Chicago, Ill.

The casein was washed in acidified water for 7 days and baked at 70–80° C. for one week.

The dextrin was prepared from corn starch, gelatinized with citric acid (20 gms. citric acid to 2 gallons water+24 pounds of cornstarch), and autoclaved under steam for 4 hours at 15–18 lbs pressure. It was then baked in an air oven for six to seven days.

The cod liver oil was provided by Mead Johnson and Company, Evansville, Indiana.

[2] The rats averaged between 40 and 60 grams in weight when placed on the experimental diets. Two males were kept in the cage with four females from the beginning of each experiment.

TABLE I.

Source of Vitamin E; Reproduction Records of Rats with Comparable Diet., Except as to Source of Vitamin E.

Diet No.	Lard	Wheat germ oil	Cod liver oil	Butter fat	No. of females	No. of living young	Probable resorptions*	REMARKS
	%	%	%	%				
4510	20	2	2	—	4	88	2	Excellent nutrition. Normal delivery.
4511	—	2	2	—	4	11	1	Two females died in parturition. Severe hemorrhage into uterus about embryos.† Bled profusely.
4488	22	—	2	—	6	130	0	Excellent nutrition. Normal deliveries. Females not mated until on diet 2.5 mos. Not kept beyond third litter. We have kept 26 females on this diet and all were very fertile.
4512	—	—	2	—	4	8	2	Three females died in same condition as 4511. Young small and pale.
4513	22	—	2	—	4	108	0	One female died at parturition with symptoms as in 4511. Nutrition apparently excellent.
4514	—	—	2	—	4	30	0	Nutrition excellent. Two females died at parturition with symptoms as in 4511.
4515	—	2	—	2	2	16	0	Short, stocky rats with rough coats. Shortage of A may have limited fertility.
4516	—	2	—	2	4	4	1	Fair nutrition. Better than in Lot 4515. One litter born after mother had been on diet 140 days.
4517	15	—	—	9	4	83	0	Excellent nutrition. Normal deliveries.
4518	15	—	—	9	4	115	0	Excellent nutrition. Normal deliveries.
4519	15	—	—	9	4	102	0	Excellent nutrition. Normal deliveries.
4520	15	—	—	9	4	130	0	Excellent nutrition. Normal deliveries.
4521	—	—	3	—	4	50	3	Three mothers died under conditions described in 4511. Excellent nutrition.‡

TABLE I (*Continued*)

Diet No.	Lard	Wheat germ oil	Cod liver oil	Butter fat	No. of females	No. of living young	Probable resorptions*	REMARKS
	%	%	%	%				
4522	22	—	3	—	4	125	0	Excellent nutrition. Normal deliveries.
4523	22	—	3	—	4	115	0	Excellent nutrition. Normal deliveries.
4524	—	—	3	—	4	9	4	Excellent nutrition. Three females died at parturition as under 4511.
4525	—	—	4	—	4	8	0	Excellent nutrition. See 4511.
4526	22	—	4	—	4	91	1	Excellent nutrition. Three females normal deliveries. Fourth had no young.
4627	22	—	4	—	4	105	0	Excellent nutrition. One female died after third litter. Symptoms as in 4511.
4528	—	—	4	—	4	25	1	Excellent nutrition. Three died at parturition as in 4511.
4529	—	—	4	—	4	19	0	Excellent nutrition, but four females died at parturition as in 4511.
4530	—	—	—	5	4	36	1	Excellent nutrition. Normal deliveries.
4534	—	—	8	10	4	54	0	Good nutrition. Normal deliveries.
4535	—	—	—	5	4	21	0	Good nutrition. Normal deliveries.
4536	—	2	—	10	4	66	1	Excellent nutrition. Normal deliveries.
4537	—	2	—	10	4	50	1	Excellent nutrition. Normal deliveries.
4538	—	—	—	5	4	14	?	Fair nutrition. Weight of 1 female fluctuated suggesting resorption.
4539	—	—	—	10	4	37	0	Good nutrition. Normal deliveries.
4540	—	—	—	10	4	62	0	Excellent nutrition. Normal deliveries.

*Probable resorption. Rats obviously pregnant. Isolated but no young seen.

†At 24 days a litter of six lost use of legs. Fore legs turned under; hind legs sprawled; eyes watery and loss of normal exophthalmos. Fur dry and rough. All but 1 died within 2 days from onset of symptoms. This condition is apparently that described by Evans and Burr (5).

‡This condition was noted only in rats receiving cod liver oil, and as a rule those without lard. Nelson, Jones, Adams and Anderegg have also noted a high mortality among females fed cod liver oil. Lard appeared to afford some protection against the injury caused by cod liver oil (4).

TABLE II.

Source of Vitamin E; Potency or Sterility in Male Rats on Otherwise Comparable Diets.

Diet No.	Lard	Wheat germ oil	Cod liver oil	Butter fat	No. of males	CONDITION AFTER DAYS
	%	%	%	%		
4510	20	2	2	—	8	5 examined at 75-189 days and 1 at 223 days, 100% motility.
4511	—	2	2	—	7	1 at 125 days 100% motility. 1 at 150 days some degeneration. 2 at 179 days mostly degenerated. 1 at 213 days mostly spermatids.
4512	—	—	2	—	8	1 at 75, 1 at 100, 1 at 125 days 100% motility. 1 at 149 days about 50-60% motility, many dead sperm and few spermatids. 1 at 179 days extensive degeneration, with few dead sperm and many spermatids. 3 at 214 days showed no motility, few dead sperm, many spermatids. 2 showed about 5% motility. A sixth male however, showed good motility, but with a few dead sperm.
4513	22	—	2	—	8	3 at 150 days 100% motility. 1 at 179 days practically no motility. Many dead sperm, few spermatids. At 214 days 4 males examined, 100% motility.
4514	—	—	2	—	8	At 150 days 100% motility (4 males); at 179 days 1 male had only dead sperm; at 214 days 2 males showed 100% motility; 1 showed about 10% motility, dead sperm and few spermatids.
4515	—	2	—	2	5	At 123 days 100% motility (2 males). 1 at 149 days about 80% motility, few spermatids. 1 at 186 days and 1 at 212 days showed 100% motility.
4516	—	2	—	2	6	1 at 101 days 100% motility. 1 at 123 days about 30-40% motility; dead sperm and spermatids. 2 at 159 days about 50% motility. 1 at 185 days 100% motility. 1 at 212 days 100% motility.
4517	15	—	—	9	7	At 220 days 100% motility (7 males).
4518	15	—	—	9	6	At 220 days 6 males showed 100% motility.
4519	15	—	—	9	7	At 220 days 7 showed 100% motility.
4520	15	—	—	9	9	At 211 days 7 showed 100% motility; 1 at 217 days complete degeneration.
4521	—	—	3	—	7	At 220 days 7 showed 100% motility.

TABLE II. (*Continued*)

Diet No.	Lard	Wheat germ oil	Cod liver oil	Butter fat	No. of males	CONDITION AFTER DAYS
4522	22	—	3	—	7	At 211 days 6 showed 100% motility; 1 showed almost complete degeneration
4523	22	—	3	—	6	At 219 days 6 showed 100% motility.
4524	—	—	3	—	7	At 183 days 2 showed 100% motility. At 209 days 4 showed only dead sperm and a few spermatids.
4525	—	—	4	—	8	At 148 days 3 showed 100% motility. At 183 days 3 showed 15-20% motility; dead sperm and spermatids present. At 209 days 2 showed complete degeneration.
4526	22	—	4	—	8	At 126 days 2 mostly motile, few dead sperm. 3 at 150 days 60-70% motility; some spermatids. At 183 days 1 showed 100% motility. 2 at 205 days 100% motility.
4527	22	—	4	—	7	At 179 days 4 showed 100% motility. 3 at 201 days 100% motility.
4528	—	—	4	—	7	At 179 days 3 males showed 100% motility. 3 at 201 days 100% motility. 1 about 80% motility.
4529	—	—	4	—	7	At 179 days 4 showed 100% motility. 3 at 201 days 100% motility.
4530	—	—	—	5	7	At 129 days 3 showed 100% motility. 1 at 148 days had only dead sperm and spermatids. 1 at 178 days showed very few motile sperm; many dead sperm and spermatids. 2 at 198 days few dead sperm and spermatids.
4534	—	—	—	10	7	1 at 75 days and 1 at 101 days showed 100% motility. 1 at 124 days about 70% motility with dead sperm and spermatids in field. 1 at 150 days no motility, only dead sperm and spermatids. 3 at 185 days complete degeneration.
4535	—	—	—	5	7	1 at 75 days 100% motility. At 100 days 3 showed dead sperm and some spermatids. At 150 days 3 showed complete degeneration.
4536	—	2	—	10	7	At 175 days 4 showed 100% motility. 3 at 125 days 100% motility.
4537	—	2	—	10	7	2 at 175 days showed 100% motility, 5 at 150 days no motility, few dead sperm and spermatids.

TABLE II. (*Continued*)

Diet No.	Lard	Wheat germ oil	Cod liver oil	Butter fat	No. of males	CONDITION AFTER DAYS
4538	—	—	—	5	7	At 70 days 1 showed only dead sperm and spermatids; 1 showed 100% motility. At 95 days 1 showed no motility, a few dead sperm and many spermatids. At 119 days 1 showed 100% motility. At 153 days 3 showed no motility, few dead sperm and spermatids.
4539	—	—	—	10	7	At 70 days 2 showed 100% motility. 2 at 95 days no motility, only dead sperm and spermatids. 3 at 119 days same as preceding.
4540	—	—	—	10	8	At 94 days 3 showed 100% motility. 2 at 131 days no motility, only dead sperm. 3 at 147 days complete degeneration.
4488	22	—	2	—	6	1 at 176 days no motile sperm; degeneration in progress. 1 at 200 days same condition. 4 at 211 days degeneration in progress, a few more spermatids.
4488	22	—	2	—	7	4 at 117 days 100% motility. 1 at 136 days 100% motility. 1 at 190 days and 1 at 229 days also showed 100% motility.

DISCUSSION

The results obtained with certain contrasting diets will be pointed out. Lot 4510 contained lard 20, wheat germ oil 2, and cod liver oil 2%. Growth curves of both sexes were excellent. There was high fertility, as shown in Table I. Lot 4511 contained wheat germ oil 2, and cod liver oil 2%. The growth curves of the males were good. The average weight was about 330 grams at 7 months, whereas those with the lard (4510) averaged about 380 grams at this age. The mortality of females in this series was high when lard was omitted from the diet (see Table I). Growth was good and the animals appeared to be in normal health until near the end of pregnancy. They had difficulty in delivery and usually died, in many cases with hemorrhage into the uterus. Throughout the series those getting lard were far superior to those without it.

A similar contrast was presented between Lots 4512 and 4513. The former received 2% of cod liver oil, whereas the latter received 2% of cod liver oil and 22% of lard. Lot 4513 was superior to 4512 in growth and

TABLE III.
COMPOSITION OF DIETS

Diet No.	Yeast	Casein	Salt mixture	Lard	Cod liver oil	Wheat germ oil	Ferric citrate	Dextrin	Butter fat	Rolled oats	$CaCO_3$
	%	%	No. %	%	%	%	%	%	%	%	%
4510	10	32	185,4.0	20	2	2	—	30.	—	—	—
4511	10	25	" 4.0	—	2	2	—	57.	—	—	—
4512	10	25	" 4.0	—	2	—	—	59.	—	—	—
4513	10	32	" 4.0	22	2	—	0.3	29.7	—	—	—
4514	10	25	" 4.0	—	2	—	0.3	58.7	—	—	—
4515	—	18	" 3.7	—	—	2	—	—	2	40	—
4516	10	20	" 4.0	—	—	2	—	62.	2	—	—
4517	10	18	" 4.0	15	—	—	0.5	43.5	9	—	—
4518	10	18	" 4.0	15	—	—	—	44.	9	—	—
4519	10	30	" 4.0	15	—	—	0.5	31.5	9	—	—
4520	10	30	" 4.0	15	—	—	—	32.	9	—	—
4521	10	32	" 4.0	—	3	—	—	51.	—	—	—
4522	10	32	" 4.0	22	3	—	—	29.	—	—	—
4523	10	32	" 4.0	22	3	—	0.3	28.7	—	—	—
4524	10	25	" 4.0	—	3	—	0.3	57.7	—	—	—
4525	10	25	" 4.0	—	4	—	0.3	56.7	—	—	—
4526	10	32	" 4.0	22	4	—	0.3	27.7	—	—	—
4527	10	32	" 4.0	22	4	—	—	28.	—	—	—
4528	10	32	" 4.0	—	4	—	—	50.	—	—	—
4529	10	32	" 4.0	—	4	—	—	49.	—	—	1
4530	10	20	50,5.6	—	—	—	—	59.4	5	—	—
4534	10	20	51,6.1	—	—	—	—	53.9	10	—	—
4535	10	20	51,6.1	—	—	—	—	58.9	5	—	—
4536	10	20	51,6.1	—	—	2	—	51.9	10	—	—
4537	10	20	50,5.6	—	—	2	—	52.4	10	—	—
4538	10	20	46,4.0	—	—	—	—	61.0	5	—	—
4539	10	20	185,4.0	—	—	—	—	56.	10	—	—
4540	10	20	50,5.6	—	—	—	—	54.4	10	—	—
4549	10	20	46,4.0	—	—	—	—	56.	10	—	—
4550	10	20	185,4.0	—	—	—	—	61.	5	—	—
4488*	10	32	185,4.0	22	2	—	—	30.	—	—	—

* This is the diet of Evans and Burr for producing sterility and resorptions.

Those diets containing ferric citrate were employed to test the hypothesis of a relation of vitamin E to iron assimilation. The results demonstrate that this view is erroneous.

SALTS No. 51		SALTS No. 185	
$CaCO_3$	1.5	NaCl	0.146
KCl	1.0	$MgSO_4$ (anhydrous)	0.225
NaCl	0.5	$NaH_2PO_4+H_2O$	0.293
$NaHCO_3$	0.7	K_2HPO_4	0.805
MgO	0.2	$CaH_4(PO_4)_2+H_2O$	0.456
Ferric citrate	0.5	Fe citrate (ic)	0.100
KH_2PO_4	1.7	Ca lactate	1.098

Salts No. 46 is the same as Salts No. 185 except that it contains no iron.

Salts No. 50 is the same as Salts No. 51 except that it contains no iron.

reproduction. This was probably due to the fact that 3 females in Lot 4512 died when giving birth to their first litters. The fourth female in this lot, however, gave birth to 4 litters.

Lot 4515 is of special interest since 40% of rolled oats, 2% of wheat germ oil, and 2% of butter fat were the sole sources of vitamin E. One male examined at 123 days, 1 at 186 days and one at 212 days all showed 100% motility of sperm cells. They were rather rough coated and not in excellent condition. Lot 4516 received 10% of yeast, 2% of wheat germ oil, and 2% of butter fat. One male examined at 100 days showed 100% motility; 1 at 123 days showed 30–40% motility. There were some dead sperm and some spermatids; 1 examined at 150 days showed 100% motility. One examined at 159 days showed about 50% motility. One at 186, and 1 at 212 days showed 100% motility. The vitamin A content of these diets was very low, but the E content was high. There were 4 females in Lot 4516; each produced but 1 litter. This may have been due to the low A content of the diet. There were 2 females in Lot 4515 and it was not until they had been on the diet six and a half months that they produced young. These rats were much under-sized and had but few young.

Lot 4518 was fed the Evans and Bishop diet consisting of casein 18%, salt mixture No. 185, 4.0%, dextrin 44, lard 15, butter fat 9, and yeast 10%. Four females produced 14 litters in 7 months. The same diet fed the previous year to ten females promoted good growth, and although the rats looked fine they had no young. Our butter, yeast and lard were from the same sources both years. We have no explanation to offer for the pronounced differences in the two experiments.

Lot 4521 and 4522 stand in marked contrast. Growth of both groups was excellent. The mortality among females was high in Lot 4521. This did not contain lard. The same description applies to these as to Lots 4510 and 4511 (see Table I).

Lot 4534 should be compared with 4518. The latter diet contained lard 15% and butter fat 9%, whereas 4534 contained only butter fat 10%. One male showed testicular degeneration at 150 days. At 185 days 5 showed complete degeneration. The males in 4518 (Evans and Bishop diet) after 212 days showed 100% motility.

We can see no other interpretation for these results than that certain samples of lard contain vitamin E, and the same must be true of certain samples of cod liver oil. It is, therefore, not possible to rely upon any fats or oils yet studied being lacking in this substance. Experimental work in this field is greatly complicated thereby, since only old and tested

samples can be known to lack E, and in these the A content will be problematical, since this principle tends to become inactive in old samples.

Our most interesting results relate to the marked degree of improvement in fertility of rats fed either cod liver oil or butter fat *together with lard*. We have uniformly observed better fertility with diets containing 2, 3 or 4% of cod liver oil than with similar ones containing 5 or 10% of butter fat. Since the same dextrin and casein were fed to males which did and those which did not grow sterile, it seems justifiable to attribute protection, where it occurred, to the fats or oils included in the diets.

Summary of Results

We have previously reported our observation that ferrous sulfate accelerates the destruction of vitamin A. Diets kept at near the freezing temperatures do not suffer this change. Our former conclusion that wheat germ oil, because of its content of E, protected animals against the injurious effect of feeding ferrous sulfate, we now interpret differently. The injury due to ferrous sulfate is the result of destruction of vitamin A. There is no evidence that vitamin E has any rôle in iron assimilation.

Certain samples of lard and of cod liver oil promote fertility to an extent which compels the conclusion that they contain considerable amounts of E.

There is considerable variation in the degree to which individual male rats withstand the effects of diets low in vitamin E. Some remain potent much longer than others. This complicates the study of the distribution or content of vitamin E.

Careful duplication of the diets of Evans and Bishop, and of Evans and Burr, produced female rats which were highly fertile in certain series, and sterile in others. This may be accounted for on the basis of differences in the vitamin E content of samples of the fats employed.

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A DETAILED STUDY OF THE ROLE OF VITAMIN B[1] IN ANOREXIA IN THE ALBINO RAT[2]

By BARNETT SURE

(Department of Agricultural Chemistry, University of Arkansas, Fayetteville, Ark.)

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AFTER a careful study of anorexia in 1471 children between the ages of 1 and 14 years, Bartlett (2) has divided his cases in two groups: (a) organic anorexia in which a definite cause for the condition could be determined; (b) functional anorexia in which an organic cause that could possibly explain the condition could not be found. The commonest pathological conditions found associated with marked loss of appetite were: (1) septic tonsils and adenoids; (2) acute infectious diseases; (3) tuberculosis; (4) pyelitis; and (5) dental caries. Thirty-five per cent of these children belonged to the group of functional anorexia and the most reliable method of treatment was the introduction of fresh calf's liver, broiled beef steak and lamb's kidneys into the diet of the child. Daily administrations of saccharated iron and cod liver oil were also tried without any significant responses. The body weight was found to be the most reliable objective criterion in the treatment of functional anorexia. It is impossible to conclude from Bartlett's investigation what specific dietary factor, if any, was responsible for the restoration of the appetite with the accompanying gains in body weight of his patients. He suggests that liver may contain a specific stimulant to the appetite.

In 1920 Karr (3) made a study of the rôle of vitamin B in food consumption in the dog and demonstrated very clearly that dogs given a diet deficient in vitamin B decrease their daily food intake until absolute inanition develops at which stages of the avitaminosis complete restoration of the appetite was obtained with a number of vitamin B containing products, but yeast, particularly brewer's yeast, proved to be the most potent source of the appetite-provoking substance. Cowgill (4) improved

[1] Recently a considerable literature has accumulated on the dual nature of Vitamin B[1]. In this paper the term "Vitamin B" is used in the sense to signify a combination of the thermolabile (anti-neuritic) and thermo-stable factors, both of which are necessary for growth.

[2] Research Paper No. 62, Journal Series, University of Arkansas. From the Department of Agricultural Chemistry, University of Arkansas, Fayetteville.

Karr's synthetic rations for the dog and made them satisfactory with respect to all known dietary factors with the exception of vitamin B. On his modified dietary regime Cowgill corroborated Karr's work in every respect. Later in cooperation with Deuel (5) Cowgill reported that 600 milligrams of a wheat embryo preparation per dog per day was adequate for a period of 3 months.

In connection with recent studies of the vitamin B requirements of nursing young (albino rat) (6) a good many mothers became available for study during the post-lactation period. A detailed investigation was begun on a representative group of individuals, ranging in age from 7 to 9 months, the purpose of the research being to determine the minimum daily dosage of various vitamin B concentrated extracts required to restore the normal appetite and body weight at the stage in the avitaminosis as near complete inanition as possible. We had access in our laboratory to a variety of potent vitamin B concentrates, all of which were prepared under the author's direction by Mr. E. H. Stuart, Chemist of the Eli Lilly Research Laboratories, Indianapolis, Ind., with the exception of the vitamin B picrate which the writer prepared in his own laboratory. A brief description of methods of preparation of the various concentrates employed in this investigation are given below. References to the literature are given for detailed methods of preparation.

F. E. Yeast represents baker's yeast adsorbed on Fuller's earth. (7)

Yeast Concentrate No. 1 is the product released from *F. E. Yeast* with sodium hydroxide and the alkali neutralized with sulfuric acid. (7)

Yeast Concentrate No. 4 is a duplicate preparation of No. 1.

Yeast Concentrate No. 16 is a product prepared from No. 1 by adsorption on silica gel and by releasing the vitamin at various hydrogen ion concentrations. (8)

Yeast Concentrate No. 18 was prepared similarly to Nos. 1 and 4 with the exception that dilute alcohol instead of acidulated water was used as the solvent.

Vitamin B picrate was prepared by precipitating the released product from *F. E. Yeast* with a methyl alcoholic solution of picric acid. (9)

The potency of the concentrates in terms of dehydrated yeast is as follows: Yeast concentrates Nos. 1 and 4 are seven times as concentrated as Northwestern Yeast (powdered yeast foam tablets secured from the Northwestern Yeast Company, Chicago, Illinois). Yeast Concentrate No. 18 is nine times as concentrated as Northwestern Yeast; and Yeast Concentrate No. 16 is eighteen times as concentrated as Northwestern Yeast. Nine-tenths of the *F. E. Yeast* product is composed of Fuller's

earth and, therefore, is not a concentrated product. We have not performed enough experiments on the vitamin B picrate to warrant a comparison of its potency in terms of dehydrated yeast.

Experimental

The following technique was used in this investigation. Nursing mothers (albino rat) at the termination of the lactation period were allowed the same vitamin B deficient ration No. 1009[3] they had during lactation, but the supplementary daily allowances of vitamin B containing materials, in the form of dehydrated yeast or concentrated extracts therefrom, were discontinued. Daily records were kept of food consumption and body weight and when marked inanition was apparent vitamin B therapy was initiated and daily observations on food intake and growth were continued. The period of vitamin therapy, depending on the amount of concentrate available, varied between 6 and 40 days. When sufficient amounts of the vitamin preparations were available the experiment was continued until complete restoration of body weight was secured.

Space does not permit to present in detail all the cases studied. Results are, therefore, summarized in Tables I and II. Since, however, no definite conception can be obtained of the rapidity of the responses secured in anorexia after vitamin B therapy without some detailed presentation of our findings, results on one case, which is typical and representative of eleven additional cases investigated, are shown in detail graphically in Chart I.

Chart I (Experiment 1204, Female No. 4905). In 15 days Female No. 4905 reduced her weight from 192 grams to 131 grams, a loss of 61 grams or 31.7% of her body weight. At the same time this animal reduced her daily food intake from 13 grams daily to nothing on the 10th day of the experimental period, and absolutely refused to eat for two subsequent days. These three days of complete inanition were followed by a sharp rise in the food consumption curve which lasted only one day during which period the animal ate 8 grams. The next day the food intake was reduced to 3 grams and the following day to 1 gram. This rise in food consumption, lasting only for one day, just before complete inanition develops we have noted with a great deal of regularity not only in the entire group of animals of this investigation, but in 24 additional ones (on which we are at present conducting experiments on effect of vitamin B deficiency on hematopoietic function) (10) and my tentative explanation of this phenomenon is that

[3] Composition of Ration No. 1009: casein (purified), 20%; agar-agar, 2%; butter fat, 5%; McCollum's salt mixture #185, 4%; and dextrin, 69%.

vitamin B is liberated through breaking down of the depots during advanced stages of catabolism. Since we are at present finding atrophic changes in the liver (11) (characterized by marked vacuolization, capillary congestion, and edomatous condition), and furthermore, since it has been demonstrated (12) that the liver is a depot for vitamin B storage, my

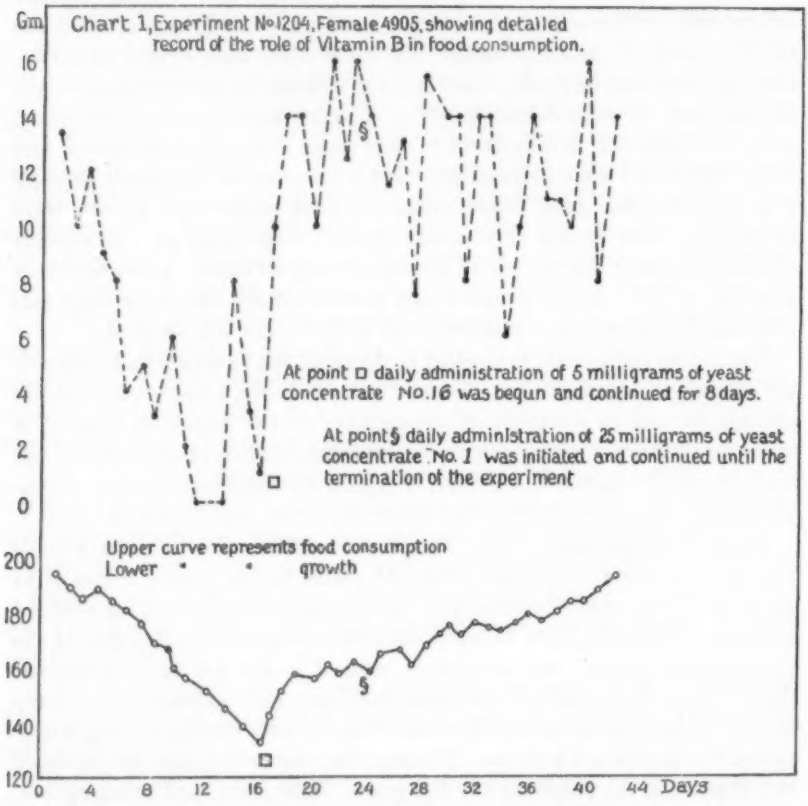
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Chart 1, Experiment No 1204, Female 4905, showing detailed record of the role of Vitamin B in food consumption.
16
14
12
10
8
6
4
2
0
§
□
At point □ daily administration of 5 milligrams of yeast concentrate No. 16 was begun and continued for 8 days.
At point § daily administration of 25 milligrams of yeast concentrate No. 1 was initiated and continued until the termination of the experiment
Upper curve represents food consumption
Lower " " growth
200
180
160
140
120
0 4 8 12 16 20 24 28 32 36 40 44 Days

suggestion is that vitamin liberated from the catabolism of the liver is the factor responsible for such temporary stimulation of food consumption just preceding complete inanition. On the 15th day, the critical point of the experiment, when vitamin therapy was begun the animal showed incoordination of the muscles of the posterior quarters with no marked signs of polyneuritis. Experience with numbers of other animals had showed us that any gradual recovery of the appetite at that stage of

avitaminosis could not be anticipated, and that when left in that state on the vitamin B deficient diet for another day or two marked symptoms of beri-beri develop characterized by complete paralysis, muscle chills, cyanosis and later respiratory failure resulting in death. Vitamin therapy was, therefore, begun on that day. We were very anxious to use in this experiment our most highly concentrated preparation. Unfortunately we had only 43 milligrams left out of a total of 14.75 grams, the rest having been consumed in studies of vitamin B requirements of nursing young. We had enough, however, of the preparation in question, No. 16, for eight daily 5 milligram dosages, and Chart I clearly shows how striking the response was. In 24 hours after receiving the first 5 milligrams dosage the animal multipled its daily food consumption by fourteen times, and the increase in daily food intake was continuous for the entire eight-day experimental period. During this short period of eight days Female No. 4905 gained 29 grams, which is over three times the gain a normal female rat makes in a week on an optimum diet during its most rapid period of growth. Because of the depletion of concentrate No. 16 we were obliged to substitute this preparation by concentrate No. 1. The daily dosage of the latter extract was increased to 25 milligrams. The continuous marked response in food consumption with the accompanying gains in weight is apparent. In 27 days the animal regained the 31.7% of the weight it lost during the 15-day period of avitaminosis.

Several cases were allowed to progress to the more advanced state of polyneuritis so that the vitamin B concentrate could not be voluntarily consumed and had to be administered with a medicine dropper. Following the vitamin administration even in such severe cases we never failed to secure marked responses in food consumption accompanied by significant increase in body weight in as short a period as 24 hours,—in some cases as early as six hours after vitamin therapy was initiated. The results on the various concentrates shown in Tables I and II are so striking as to preclude any discussion. As small a daily dosage as 5 milligrams of the vitamin B picrate, equivalent to 2.5 milligrams of vitamin,[4] produced marked responses in food consumption within 24 hours[5] which was continuous during the entire experimental period.

Plimmer and Rosedale (13) have recently suggested that the diet of the people of England is deficient in vitamin B and that a study of the daily

[4] One-half of the preparation is picric acid and harmless to the animal in such small amounts.

[5] Record not shown in Table I for lack of space.

TABLE I.

EFFECT OF ADMINISTRATION OF CONCENTRATED PREPARATIONS OF VITAMIN B ON FOOD CONSUMPTION

Experiment number	Animal number	Period of vitamin depletion	Period of vitamin therapy	Source of vitamin used for therapy*	Amount of vitamin administered	Average Daily Food Consumption				
						Last 4 days of depletion period	During 4 days following therapy period	During entire depletion period	During entire therapy period	Increase in average daily food consumption
		days	days		mgms.	gm.	gm.	gm.	gm.	per cent
1010	4498	27	17	F. E. Yeast	250	3.2	18.2	7.4	13.6	83.8
1010	4499	38	17	F. E. Yeast	250	2.7	11.7	7.0	12.1	67.8
1009	4497	26	23	F. E. Yeast	250	3.0	11.0	8.3	11.3	36.1
1013	4503	29	16	F. E. Yeast	100	3.7	12.5	7.3	10.7	46.6
1012	4502	22	24	Yeast Concentrate No. 4	10 to 40	3.7	11.5	6.5	11.6	76.9
1200	4897	34	40	Yeast Concentrate No. 1	50	3.4	10.0	6.0	8.2	27.3
1205	4906	20	30	Yeast Concentrate No. 1	10 to 25	6.5	10.5	7.2	9.6	33.3
1011	4501	38	13	Vitamin B picrate	20	3.2	8.5	7.5	9.6	33.8
1012	4502	19	6	Vitamin B picrate	5	4.2	7.7	5.2	7.8	85.7
			7	Yeast Concentrate No. 10	5		7.7	5.2	8.1	92.9
1203	4903	29	40	Yeast Concentrate No. 18	25	2.2	11.0	6.1	9.0	47.5
1196	4888	18	26	Yeast Concentrate No. 18	5 to 10	4.5	10.5	6.9	10.3	49.3
1204	4905	15	8	Yeast Concentrate No. 16	5	3.0	12.0	5.5	12.0	118.0
			16	Yeast Concentrate No. 1	25	—	11.5	5.5	13.1	138.2

* For methods of preparation of vitamin concentrates see text.

TABLE II.

EFFECT OF ADMINISTRATION OF CONCENTRATED PREPARATIONS OF VITAMIN B ON GROWTH

Experiment number	Animal number	Period of vitamin depletion (days)	Period of vitamin therapy (days)	Source of vitamin used for therapy*	Amount of vitamin administered	Weight (in Grams)				
						Beginning of experiment	On day vitamin therapy was initiated	On fourth day after vitamin therapy was begun	On day experiment was terminated	Increase in wgt. during vitamin therapy period
					(mgms.)					(gm.)
1010	4498	27	17	F. E. Yeast	250	204	143	168	192	49
1010	4499	38	17	F. E. Yeast	250	188	117	144	174	57
1009	4497	26	23	F. E. Yeast	250	208	132	152	187	55
1013	4503	29	16	F. E. Yeast	100	107	130	150	162	32
1012	4502	22	24	Yeast Concentrate No. 4	10 to 40	203	129	149	198	69
1200	4897	34	40	Yeast Concentrate No. 1	50	226	118	136	182	64
1205	4906	20	30	Yeast Concentrate No. 1	10 to 25	160	139	150	176	37
1011	4501	38	13	Vitamin B picrate	20	222	133	153	171	38
1012	4502	19	6	Vitamin B picrate	5	170	124	132	138	14
			7	Yeast Concentrate No. 10	5		132	145	152	20
1203	4903	29	40	Yeast Concentrate No. 18	25	208	141	160	210	69
1196	4888	18	26	Yeast Concentrate No. 18	5 to 10	206	174	185	205	31
1204	4905	15	8	Yeast Concentrate No. 16	5	192	131	160	160	29
			16	Yeast Concentrate No. 1	25	—	160	168	191	31

* For methods of preparation of vitamin concentrates see text.

food intake shows that insufficient vitamin B is eaten to balance the large quantities of white flour, sugar, and margarine. These investigators, therefore, maintain that shortage of vitamin B is closely connected with the origin of the common gastro-intestinal troubles of the present day. Such contention is precisely in agreement with that expressed by McCarrison (14) some years ago.

Since the calorific intake of the American people has as its source mainly degerminated cereals, sugar, and meat, and since their diet is not balanced by sufficient amounts of fresh vegetables[6] (15) to insure an optimum vitamin B intake, it is suggested that the organic and functional anorexias so frequently encountered in the human may largely be attributable to cumulative effects of vitamin B deficiency.

Summary

1. Vitamin B deficiency is a determining factor in the development of anorexia.

2. Complete anorexia associated with vitamin B deficiency can be entirely cured with vitamin B therapy.

3. The response to vitamin therapy in anorexia is most marked when highly concentrated preparations of vitamin B are employed.

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15. "Wheat Studies of the Food Research Institute," Stanford University, 1926, ii, 274, Table V, adapted from Pearl, R., *The Nation's Food*, Philadelphia, 1920, 236.

[6] Fresh vegetables contain large amounts of water (90 to 95%), and it is necessary to consume an abundance of such products to insure an optimum vitamin B intake for all physiological functions. For instance, we have recently found in our laboratory that as much as 30 grams of fresh lettuce per nursing rat per day, equivalent on the dry basis to 15% of total food intake, did not furnish sufficient amount of vitamin B for normal physiological function of milk secretion.

THE CHARACTER OF ENERGY METABOLISM DURING WORK[1]

M. ELIZABETH MARSH
(From the Department of Vital Economics, University of Rochester, Rochester, N. Y.)

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THE recent researches of Fletcher and Hopkins (1), of Meyerhoff (2), and of Hill (3), support the old hypothesis of Chauveau (4) that only carbohydrate supplies the energy in muscular work. While much of the recent work has been done upon isolated muscle of cold-blooded animals, Hill and his students have undertaken studies upon the human subject. In Furusawa's (5) experiments, made upon two men, in which the work consisted of short periods of running-in-place, the R.Q. of the excess metabolism was for short periods in the neighborhood of unity. When the exercise was continued for longer periods, the quotient of the excess metabolism fell. When the subject was on a high fat diet, the excess quotient remained in the neighborhood of unity for only two or three minutes of exercise and then decreased as the work was continued. Hill and his pupils have expressed an emphatic belief that before it can yield energy, fat must first be transformed to carbohydrate, the latter substance as lactic acid, being a specific fuel for the peculiar mechanism of muscle. Lusk (6) has challenged this theory, pointing out the paucity of evidence for the transformation of fat to carbohydrate in the animal body. Henderson and Haggard (7) in their work on the Yale crew have furnished evidence, similar to that developed by Zuntz (8) and his pupils in answer to the hypothesis of Chauveau, to indicate that both fat and carbohydrate furnish energy for muscular work in relatively the same proportion as they furnish energy during rest just before the work. Himwich and Rose (9) from the evidence obtained by analysis of the arterial and venous blood of single muscles artificially stimulated in anesthetized animals report that the level of the R.Q. of the exercising muscle is dependent upon the initial level, that it changes hardly at all from this level and that carbohydrate is not the only substance oxidized. Hill has rested his case strongly upon the older experiments of Krogh and Lindhard (10) showing a difference in efficiency of human muscles in subjects subsisting upon

[1] Presented to the University of Rochester as part of a thesis in partial fulfillment of the requirements for the Ph. D. degree.

high carbohydrate diet on the one hand and on high fat on the other. While the Danish observers believe strongly in the possibility of a transformation from fat to carbohydrate, as well as the reverse change in the animal body, they are careful to point out that their experiments do not prove such transformation. Their observations regarding the respiratory quotient may be summarized in this way. When the initial quotient was above 0.90 the quotient for the work period fell on the average 0.05, and when the quotient was below 0.80, it rose during the work period on an average 0.03. When the initial quotient was between 0.80 and 0.90 the average change during work was only slight. They put forward the hypothesis that "the proportion of carbohydrate to fat metabolized is a function of the relatively available quantities of the two substances and substantially the same during rest as during muscular work". Whether carbohydrate is transformed to fat, or fat to carbohydrate, depends in their view upon which is in excess at any given amount. Crude quotients above 0.90, they believe, may include the processes concerned in the transformation of carbohydrate to fat and quotients below 0.80 may include processes concerned in the transformation of fat to carbohydrate. These observations regarding the changes in the quotient during work have been confirmed by Boothby and Barborka (11) and are to be seen in the more recent work of Henderson and Haggard (7).

The issue therefore once more is squarely joined between the view that carbohydrate is the sole source of energy in muscular work and the view that fat equally with carbohydrate may furnish its energy directly without transformation into carbohydrate. A crucial experiment which will give a positive answer as between these two theories is difficult to devise. The R.Q.'s are necessarily merely the resultant effect of the total oxidation and reduction processes going on in the tissues. The falling of the quotient following the high fat feeding may be given by direct combustion of the fat or by transformation of the fat first to carbohydrate and then its combustion as such. But if after heavy fat feeding the composite quotient falls just as much in muscular work when there is every reason for the transformation of fat to carbohydrate as it does in muscular rest when, according to the newer views, carbohydrate is not needed to supply lactic acid for its peculiar function in muscular contraction, the common-sense interpretation would seem to be that the governing factor is relative availability or relative concentration of fat in the circulation. Experiments of this nature to be complete should demonstrate no change in carbon dioxide combining power or, if acidosis is found, it should be neutralized. A low R.Q. persisting under these conditions would show at least that a rise of

quotient which so often has been reported after a low initial quotient has no significance with reference to the predilection of muscles for carbohydrate. If, now, in addition we find that a high carbohydrate diet causes just as much rise in quotient during work as during rest and no more, the conclusion would be almost inevitable that this foodstuff is oxidized in larger proportion in muscular work for the same reason that it is during rest. This seems to be as near as we can come to a crucial test of the problem so far as direct observations on working subjects are concerned. Collaterally many arguments may be assembled on both sides of the question. In the writer's opinion the weight of evidence is against the transformation of fat to carbohydrates in higher animals under normal conditions, but the present paper will be devoted entirely to a presentation of evidence obtained from work experiments.

Experimental

The experiments reported in the present paper grew out of a plan to study the muscular efficiency of children. After a few experiments had been made upon several boys it was realized that the results obtained on the R.Q. during work were quite contrary to the views of Hill (3), and particularly to the results of Furusawa (5), mentioned above. Were these differences due to difference of technique, or what additional precautions must be taken to insure positive results in short-period work experiments? It was deemed necessary to investigate carefully all points of the technique, particularly the factors concerned in the production of the R.Q. for the excess metabolism during work before going on with the original plan.

Most of the experiments have been performed on three subjects, two boys 14 and 15 years of age respectively at the start, and a young man 21 years of age. A few experiments are reported upon three other boys 15 years of age and eleven other adults varying from 21 to 31 years. The list of subjects with the number of experiments for each, their ages, heights and weights, is given in Table 1. The boys came to the laboratory in the afternoon following school and sometimes on Saturday morning. With one subject (W. M.) no attempt was made to control the diet or activity outside the experimental period in any way. The second boy (E. T.) for a part of the experiments (3 to 17 inclusive) ate a standard breakfast and lunch on the experiment day. Both were very conscientious and reliable subjects who rode bicycles more or less regularly but were of entirely different physical types. As seen in Table 1, the first subject (W. M.) was unusually tall for his age while the second (E. T.) was somewhat under-

size. The experiments were planned for a study of the relative efficiency of boys and men and also for boys of different type. The two extreme types were chosen for this purpose.

TABLE 1.

Subject	Occupation	No. of Expts.	Age	Hgt. cm.	Wgt. kg.
W.M.	High School Student	18	15	182	69.6
E.T.	"	49	14	161	52.8
T.B.	"	1	15	162	51.8
R.D.	"	1	15	164	48.5
M.W.	"	2	15	183	68.8
J.P.	Laboratory Worker	4	25	171	64.0
R.S.	"	4	23	167	69.8
C.M.	"	2	29	171	64.0
P.G.	"	1	23	170	67.0
D.M.	Medical Student	1	24	175	67.0
E.M.	"	2	21	175	70.0
J.J.	"	1	24	179	70.6
B.J.	"	1	26	177	80.4
P.H.	"	2	31	175	84.0
F.H.	"	2	23	185	70.0
J.L.	"	1	23	171	81.8
B.C.	Professional Boxer	51	21	155	56.8

The third subject (B. C.) gave his full time to the experimentation for a period of ten weeks. He was a professional boxer, well muscled and in good training generally although not specifically trained for bicycle riding. Because of his good physical condition and his splendid cooperation and interest in the experiments he was an excellent subject. He willingly underwent all the unpleasant experiences of a high fat diet for the first period of eleven days and then a second period of seven days in succession as well as other restrictions on his liberty.

APPARATUS AND METHOD

For measurement of the work done two different bicycle ergometers were used. The first one which was constructed in the laboratory is of the Prony brake type and is described by Morrison and Gaebler (12). The second one was a Krogh ergometer which Professor Lusk very kindly loaned to us. An ergometer of the type has been found by Benedict (13) to measure the work accurately to within 0.5%. In the efficiency experiments to be reported in a later paper it was found that with the same subject (E. T.) 16 experiments gave an average efficiency of 22.5% on the

Krogh ergometer and in 21 experiments on the Prony ergometer the average efficiency was 22.3%. The two machines therefore were about equally serviceable for the experiments in hand. The amount of work chosen was such as could be endured easily by the subject for a period of ten to fifteen minutes without undue fatigue or discomfort either from the more or less rigid position necessitated by the use of the respiratory apparatus, or from the mouthpiece and nose clip. The method of including the recovery period with the work period in order to get a complete record of the respiratory exchange occasioned by work, which was introduced by Hill and his associates has everything to commend it and accordingly was adopted for these experiments. As will be seen it is quite as necessary to secure recovery of the original rate of CO_2 elimination as to secure recovery of the original rate of oxygen absorption and much time had to be devoted in these experiments to the determination of the proper length of recovery for both purposes. For the efficiency study, as will be seen in a later paper, no great difference is made whether recovery is or is not quite complete, but for observations on the character of the material furnishing energy, results would not be accurate if recovery was not complete, or if, perchance, a diminished respiratory exchange followed immediately upon complete recovery and the recovery period should include this depression of metabolism.

For all these reasons it was necessary to keep the amount of work very moderate both as regards intensity and time. For the boys it was made very light, between 0.7 and 0.8 Cals. per minute for five minutes during the majority of experiments, although in a few it was continued for ten minutes and in two or three for as much as fifteen minutes. Another reason for choosing a moderate amount of work was that much younger boys and also girls could then be compared with older boys and men on the same quantitative basis. For the one adult subject (B. C.) the work was increased to 1.40 Cals. per minute for most of the experiments but was reduced to 1.1 Cals. for the last twelve experiments. The reason for this will appear in time.

The respiratory exchange was obtained with a Benedict Universal respiration machine the subject being connected to it, through a spirometer supported on a separately movable stand, by means of a mouth piece. Several types of nose clip were available in the laboratory and the subject chose the one which seemed to him most comfortable. Oxygen was admitted to the system from a large cylinder and after reduction of the pressure was measured by an accurate water meter. Two large (2 qt.) jars of soda lime were used to complete the absorption of the CO_2 at the

rate of ventilation which the blower was supplying, namely 40 liters per minute.

The apparatus was checked routinely for tightness before each experiment and the efficiency of the absorbers was established by special tests. To make certain that the sulfuric acid in the first Williams bottles was taking out all the moisture in the air before it reached the soda lime jars a U-tube containing sulfuric acid and pumice stone was inserted in the circuit so that a few liters of air could be diverted through the U-tube of acid on its way to the soda lime. The U-tube was weighed before and after to detect any gain in moisture. As a matter of fact the acid in the Williams bottles was changed sufficiently often so that no moisture was ever detected in this tube. Alcohol checks were run at regular intervals by inserting a small flame into a small chamber built into the circuit between the spirometer and the three-way valve connecting the mouth-piece to the circuit. The results are contained in Table 2 and show an average value, which is exactly the theoretical value for the combustion of alcohol, namely 0.667.

TABLE 2
ALCOHOL CHECKS

Date	R.Q.	Date	R.Q.
10-24-25	0.664	11-24-26	0.660
11-12-25	0.676	12- 6-26	0.670
2-23-26	0.673	1-13-27	0.665
3- 8-26	0.688	2- 4-27	0.665
" " "	0.674	" " "	0.671
3-20-26	0.650	3- 8-27	0.658
5-29-26	0.669	3-16-27	0.651
9-30-26	0.670	3-23-27	0.671
		Average	0.667

In the earlier experiments a record of the respirations was obtained by fastening a pneumograph around the chest of the subject and connecting this with a tambour and lever writing upon a small kymograph. A record obtained in this manner was not entirely satisfactory because the pneumograph occasionally slipped during the work period unless special precautions were taken by means of shoulder straps and other fastenings to prevent its shifting from one position on the thorax to another. Furthermore, a change of type from thoracic to abdominal respiration after work was several times noted and for this reason the pneuomgraph about the

chest failed to make an accurate record. Indeed the record made it appear that an apnea had occurred during work. In all the later experiments a record of respirations was obtained directly from the movements of the spirometer. A thread was attached to the cord suspending the spirometer and was brought down over a small pulley and fastened to a rod to which a light lever was attached. The spirometer was large enough so that for the usual resting period it was not necessary to introduce any additional oxygen and by using a long paper kymograph a record could be obtained showing the gradual fall in the bell as the oxygen was used up. Employing a time marker record as a base line the slope of the respiration record showed the rate of oxygen absorption. During the work period the oxygen absorption was so rapid that oxygen was put into the system continuously, an attempt being made to keep the spirometer level high. For this reason nothing could be learned of the rate of oxygen absorption directly from the record. But during the recovery period after the first few minutes the oxygen could be shut off completely for a time so that the slope made by successive individual respirations,

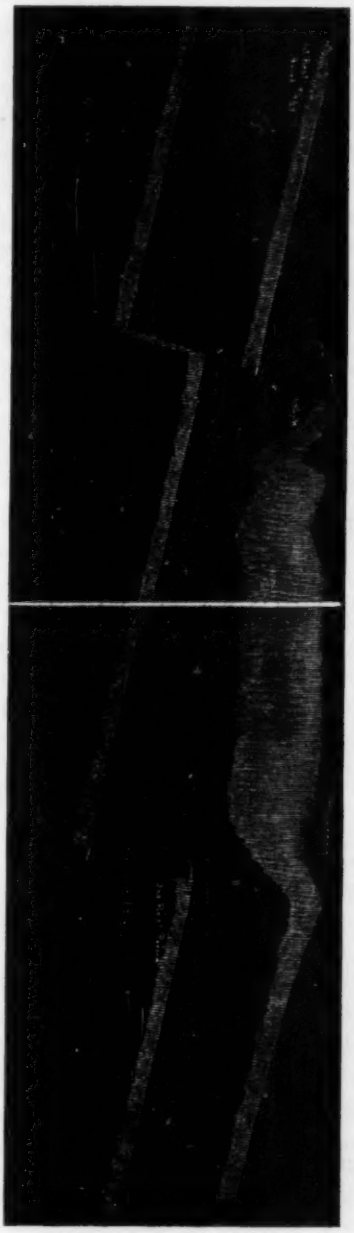

FIG. 1. Record of the respirations during rest, work and recovery and subsequent rest periods. Recovery of the rate of metabolism after work is indicated by a similar slope in the record to that of the previous resting period.

compared with the slope obtained in the earlier rest period could serve as an index of the recovery, (see Fig. 1). No attempt was made to calibrate these records to read from them the actual amount of oxygen used because this value was obtained from the meter readings with necessary corrections for temperature, water vapor and barometric pressure.

The routine procedure of an experiment was as follows: Upon coming into the laboratory the subject rested from twenty minutes to half an hour to insure recovery from previous activity. He then took his position upon the bicycle, adjusted the mouth piece and nose clip, tested for leaks around both and continued to breathe through the three-way valve into the room. When breathing had become regular and normal the valve was turned connecting the subject with the respiration machine as exactly as possible just at the end of the expiration. The first resting period was run for seven to ten minutes and the valve turned again exactly at the end of an expiration. The graphic record, of course, aided in hitting this exact point in ending the period. The subject removed the nose clip and mouth piece and usually sat in a chair while the train of absorbers was being weighed. Then followed the joint work and recovery period. As the valve was turned connecting the subject with the apparatus, he began pedaling, keeping a constant rate by means of a metronome. At the end of a certain time the subject was told to stop work but the recovery period was continued uninterruptedly with the work period. Immediately following this work and recovery period in the later experiments a second rest period was run as a check to make sure that the CO_2 and O_2 levels had returned to the original resting level.

In the experiments on subject B. C. and in the second series on E. T., nitrogen in the urine was determined and also CO_2 analysis of alveolar air was made. For the nitrogen a first collection of urine was made at the end of the first resting period and covered usually a period of 30 to 45 minutes. A second collection was made at the end of the second resting period and covered an approximately equal length of time inclusive of the work and recovery period. For the alveolar air a sample was taken after the preliminary rest, a second just before the work period was begun, a third at the end of the work and recovery period and a fourth at the end of the second resting period. The Haldane apparatus was used to determine the CO_2.

In all of the tables which follow the R.Q.'s are the quotients of the total exchange and not the non-protein R.Q.'s, even in the experiments in which nitrogen determinations were made. The R.Q. of the excess metabolism was obtained by deducting from the total CO_2 and O_2 of the work and

recovery period, the amount of CO_2 and O_2 obtained in the previous resting period calculated at the same time interval. Always the first resting period has been used as the base line rather than the average values obtained for the period before work and the period following. It was found that no material difference was made in the average values whether the first only or both of these resting periods was used as a base line.

The R.Q. as an Index of the Character of the Metabolism During Work

The R.Q. furnishes a reliable index of the character of the material furnishing energy at any given time only provided certain precautions are taken. It is the common observation of workers in basal metabolism that very often in the first determination made upon a patient there is an excess amount of CO_2 given off. The mouth piece and nose clip or the consciousness of the respirations together with possibly a slight apprehension may combine to produce overbreathing. This over-ventilation, called also "blowing off" or "Auspumpung," is even more likely to occur during the early stages of muscular work. But if the recovery period is sufficiently long, a period of retention of CO_2 would in time balance the blowing off period, and thus restore the equilibrium. It is essential that recovery may be continued to this point as well as to the point where the rate of oxygen absorption which prevailed previous to the work has been restored.

The R.Q. of the excess metabolism is the fraction in which we are most interested at present. This idea that the excess O_2 intake and CO_2 output over the basal values would more correctly represent the change taking place in the character of the metabolism occasioned by work was suggested by Benedict and Cathcart (14), although it was applied by them only to the quotients obtained after the work period and not in the actual work period itself. It was made use of by Campbell, Douglas and Hobson (15) and also by Furasawa (5). Theoretically it seems a very sensible method of getting a picture of the true character of the metabolism stimulated by work. If the muscles utilize the material for the same purpose as the entire body does during rest, and if the metabolism of the organs made active secondarily by muscular work does not differ in quality from their metabolism during rest, then the ratio of the excess CO_2 to O_2 in work should give a true picture of the altered metabolism. If the combustion of carbohydrate is increased, the excess quotient ordinarily will be higher than the quotient in the previous rest period and the excess quotient of 1.0 would indicate that the muscles used for their work energy only

carbohydrate. Practically it may not always come out according to theory. Professor Hill (16) has recently found that in sprinting (100 yd. dash) the quotients of the entire respiratory exchange for the run and the recovery therefrom was 1.00 which gave a quotient for the excess metabolism of 1.4. Ordinarily such a quotient as this indicates conversion of carbohydrate to fat. In this instance it indicates merely the uncompensated effect of over breathing as a result of the over strenuous exercise. According to Professor Hill's statement recovery from 36 seconds of violent exercise requires at least 20 minutes, and the R.Q. during a recovery period may fall as low as 0.59 which denotes retention of CO_2 to counterbalance the previous excessive elimination. It seems quite obvious that if any reliance is to be placed on the excess R.Q. the recovery of CO_2 must be established as well as of O_2. Other very high R.Q.'s found in the literature during work periods may be due to this cause.

Experiments on Normal Diet

In all something over 100 experiments have been made upon subjects who were subsisting at the time upon normal diet. In all but the first twenty-one of these the determinations of the R.Q. directly after the work and recovery period were made in addition to the resting period immediately preceding the work and recovery period. The results of these experiments are given in Tables 3 to 7 and are arranged according to subjects, rather than according to chronological order. They give, in addition to the R.Q.'s, the CO_2 and O_2 values per minute of the two resting periods in order to show whether recovery had or had not been complete. The total work in Calories and the length of the work period and recovery are likewise shown.

In the first twenty-one experiments upon nine subjects the only criteria used to determine recovery were the pulse rate and the rate and depth of respiration. The experiments will be found to be the first twelve in Table 3, the first two in Table 4 and the first seven in Table 6. The R.Q.'s of the work and recovery period combined were in some instances lower than in the preceding resting period, in some instances it was higher and in still others it remained the same. The R.Q. of the excess metabolism varied in these early experiments all the way from 0.71 to 1.04. The amount of work done and the length of the work period also varied considerably. If the experiments were of the running-in-place type rather than ergometer experiments, the subject ran for one minute while breathing into the apparatus and then stood still for nineteen minutes while continuing to breathe into the apparatus. This is a duplication of one of

TABLE 3. R.Q.'S AND CO_2/O_2 VALUES FOR DIFFERENT SUBJECTS

Subj.	Date	Expt.	Work (Cal.)	Work Period	Recov. Period	R.Q.				CO_2/O_2—cc/min.	
						Before Work	Work and Recov.	After Work	Excess Metab.	Before Work	After Work
				min.	min.						
M.W.	1-19-26	1	6.63	10	11	.80	.82	—	.83	245/308	—
"	2- 4-26	2	5.85	10	10	.85	.81	—	.76	287/339	—
T.B.	12-12-25	1	7.35	10	10	.94	.92	—	.90	250/267	—
R. D.	12-19-25	1	6.35	10	12	.82	.71	—	.79	237/290	—
D. M.	12-15-25	1	3.84	10	12	.88	.88	—	.88	293/333	—
E. M.	1-12-16	1	7.18	10	10	.83	.83	—	.83	282/339	—
"	2- 2-26	2	5.73	10	10	.92	.80	—	.72	254/275	—
C. M.	1-12-26	1	S. R.*	1	19	.82	.82	—	.82	275/334	—
"	1-20-26	2	S. R.	1	19	.89	.89	—	.89	292/327	—
J. P.	12-10-25	1	7.06	10	10	.86	.89	—	.93	231/272	—
"	12-21-25	2	7.23	10	13	.78	.79	—	.80	218/278	—
"	1- 9-26	3	S. R.	1	19	.85	.87	—	1.04	241/282	—
"	4- 1-26	4	3.92	5	9	.88	.86	.85	.84	254/288	238/280
P. G.	1-23-26	1	S. R.	1	19	.93	.94	.86	.96	259/279	264/306
R. S.**	4- 7-26	1	3.67	5	8	.75	.72	.68	.69	230/307	204/298
"	4- 9-26	2	3.04	5	8	.79	.77	.73	.75	238/303	240/327
"	6- 2-26	3	3.79	5	9	.80	.75	.74	.70	249/312	240/322
"	6- 3-26	4	3.53	5	9	.89	.83	.89	.78	250/282	245/274
J. J.	11-18-26	1	S. R.	1	19	.81	.85	—	.99	244/300	—/299
B. K.	11- 2-26	1	S. R.	1	19	.89	.83	.88	.74	247/277	.24/280
P. H.	11-11-26	1	S. R.	1	19	.84	.87	.84	1.04	266/318	260/310
"	11-11-26	2	3.64	5	7	.87	.90	.94	.95	264/305	269/285
F. H.	11-16-26	1	4.02	5	10	.90	.84	.84	.78	286/319	283/338
"	11-20-26	2	3.24	5	12	.90	.93	.89	.95	280/310	288/322
J. L.	11-23-26	1	2.73	5	10	.82	.80	—	.77	234/287	—

*S.R. = Standing Running. **First two expts. were made in post-absorptive condition.

TABLE 4. R.Q.'S AND CO_2/O_2, SUBJECT E. T.

Date	Expt.	Work (Cal.)	Work Period	Recov. Period	R.Q.			CO_2/O_2—cc/min.			Nutritive Condition
					Before Work	Work and Revoc.	After Work	Excess Metab.	Before Work	After Work	
			min.	min.							
12–5–25	1	6.89	10	10	.81	.77	—	.73	237/292	—	4 hrs. after breakfast
1–29–26	2	6.26	10	10	.87	.87	—	.87	254/291	—	3½ hrs. after lunch
2– 9–26	3	3.03	4	6	.89	.87	.82	.84	249/278	248/304	4 hrs. after lunch
2–11–26	4	3.78	5	10	.86	.83	.90	.81	234/272	235/261	4 hrs. after lunch
2–12–26	5	3.77	5	15	.87	.87	.99	.87	241/276	242/257	5 hrs. after lunch
2–16–26	6	3.23	5	15	.81	.82	.92	.83	242/299	243/265	5 hrs. after lunch
2–18–26	7	3.63	5	5	.88	.77	.89	.69	252/287	254/285	5 hrs. after lunch
2–19–26	8	3.61	5	5	.83	.78	.91	.74	239/289	257/283	5½ hrs. after lunch
2–22–26	9	3.56	5	10	.93	.85	.95	.74	276/296	271/284	4 hrs. after lunch
2–23–26	10	3.65	5	10	.88	.85	.93	.81	264/300	261/282	4 hrs. after lunch
2–25–26	11	3.55	5	7	.90	.86	.84	.82	260/291	255/303	4 hrs. after lunch
2–26–26	12	2.30	3½	8	.93	.88	.94	.81	256/275	254/270	4 hrs. after lunch
3– 2–26	13	3.64	5	8	.91	.81	.86	.69	275/301	253/296	4 hrs. after lunch
3– 4–26	14	3.56	5	9	.90	.85	.94	.79	282/314	266/282	3½ hrs. after lunch
3– 9–26	15	3.86	5	8	.91	.79	.74	.68	256/281	246/332	4 hrs. after lunch
3–11–26	16	3.77	5	8	.88	.85	.84	.80	247/280	243/286	4 hrs. after lunch
3–12–26	17	3.58	5	8	.92	.80	.88	.68	272–297	267–302	5 hrs. after lunch
3–13–26	18	3.43	5	8	.83	.80	.96	.77	277/334	266/276	1 hr. after breakfast
4–13–26	19	4.37	5	8	.84	.78	.83	.70	306/364	303/366	4 hrs. after lunch
5–12–26	20	3.57	5	10	.80	.79	—	.77	240/300	243/—	5 hrs. after lunch
5–15–26	21	3.83	5	10	.82	.80	.80	.76	255/309	240/299	4 hrs. after breakfast
5–20–26	22	3.34	5	8	.86	.81	.80	.76	229/266	224/277	4½ hrs. after lunch
5–25–26	23	3.62	5	8	.83	.81	.96	.77	240/288	235/246	4½ hrs. after lunch

TABLE 4. (*Continued*)

5-27-26	24	2.61	5	8	.80	.81	.86	.82	215/269	225/254	4 hrs. after lunch
5-29-26	25	3.86	5	15	.95	.87	.82	.73	290/307	235/287	2 hrs. after breakfast
6- 3-26	26	3.93	5	15	.81	.81	.76	.81	232/286	212/278	4 hrs. after lunch
6- 4-26	27	3.62	5	20	.82	.82	.85	.82	239/292	225/264	4 hrs. after lunch
6- 5-26	28	4.00	5	20	.82	.79	.76	.73	236/288	231/305	3 hrs. after breakfast
6- 7-27	29	3.81	5	20	.93	.87	.87	.74	258/278	242/277	4 hrs. after lunch
6-10-26	30	3.90	5	20	.92	.81	.81	.65	231/251	214/265	4 hrs. after lunch
6-11-26	31	3.71	5	20	.93	.84	.80	.68	255/275	226/284	4 hrs. after lunch
6-12-26	32	3.53	5	20	.89	.87	.90	.83	250/280	231/257	3 hrs. after lunch
6-15-26	33	3.68	5	20	.94	.95	.98	1.00	259/277	241/245	2½ hrs. after lunch
6-16-26	34	3.56	5	20	.84	.88	.85	.98	249/298	239/280	1½ hrs. after lunch
6-16-26	35	3.58	5	20	.92	.87	.80	.77	247/269	228/284	4 hrs. after lunch
6-17-26	36	3.87	5	20	.88	.84	.84	.77	236/270	228/271	3 hrs. after lunch
6-17-26	37	3.75	5	20	.92	.84	—	.70	253/276	253/—	1½ hrs. after lunch
6-18-26	38	1.27	5	19	.89	.83	.83	.80	244/274	219/264	3 hrs. after breakfast
Average*		3.79			.87	.83	.87	.78	252/289	243/282	
1- 5-27	39	3.35	5	8	.82	.85	.80	.87	239/290	253/315	5 hrs. after lunch
1—6-27	40	3.43	5	10	.94	.89	.93	.84	256/273	264/285	4½ hrs. after lunch
1-12-27	41	3.95	5	10	.96	.86	.88	.76	287/295	267/303	4 hrs. after lunch
1-15-27	42	3.54	5	8	.97	.90	.89	.81	280/290	257/290	4 hrs. after lunch
1-17-27	43	3.52	5	8	.85	.92	.85	.99	223/263	238/281	5½ hrs. after breakfast
1-19-27	44	3.40	5	8	.99	.93	.93	.87	239/251	255/274	4 hrs. after breakfast
1-20-27	45	3.42	5	8	.89	.91	.94	.94	251/282	252/268	5 hrs. after lunch
1-21-27	46	3.20	5	8	.87	.93	.86	1.03	247/284	246/285	3½ hrs. after breakfast
2- 3-27	47	3.37	5	9	1.00	.94	.92	.90	267/268	247/267	4 hrs. after lunch
2- 5-27	48	4.48	5	10	.84	.86	.81	.89	239/286	248/306	4 hrs. after breakfast
2-12-27	49	4.49	5	10	.84	.83	.87	.81	223/265	240/275	5 hrs. after breakfast
Aver		3.64			.91	.89	.88	.88	250/276	251/286	

* Omitting Expt. 38.

Furusawa's (5) experiments in which there would seem to be no question that the recovery period was sufficiently long. In Table 3, beginning with the 4th experiment on J.P. are listed a considerable number of other experiments upon adults which differed from the earlier experiments just mentioned in that, in the first place, the work was of shorter duration, either five minutes pedaling on the bicycle or again the one minute of running-in-place, and in the second place a determination was made of the CO_2 and O_2 exchange in the second resting period. There is to be noted here the same variable response to work that was found in the earlier experiments. In the case of the seven standing-running experiments four showed a rise in the work quotient with an R.Q. of the excess metabolism amounting to 1.04, 0.96, 0.99 and 1.04 respectively and one showed a fall making the R.Q. of the excess metabolism, 0.74. The other two were done on the same subject C. M. and both gave a quotient during the work and recovery period which was exactly the same as it had been previous to work, namely 0.82 and 0.89 respectively.

Determination of Time Necessary for Recovery

In view of the irregularities found in the earlier experiments, differences not only between different individuals, but between different experiments with the same individual (see Table 3) and likewise because of the uncertainty of the recovery period, it was deemed necessary to work out carefully the length of time required for complete recovery on both O_2 and CO_2 for a given piece of work, the conditions being normal throughout. For this purpose one of the boys, E. T. came regularly to the laboratory. An attempt was made to control the diet by having him eat a standard breakfast and lunch each experiment day, but the time elapsing from the last meal to the time of the determinations was not always the same, consequently there was still considerable variation in the resting quotients previous to work. The work was reduced to five minutes duration and had an average value of 0.76 Cals. per minute. Recovery periods of five, eight, ten, fifteen and twenty minutes were then tried. A second resting period was run directly after the work and recovery period but the excess metabolism was still calculated from the base line as given by the first resting period.

The individual experiments in this series are listed in Table 4, experiments 3 to 37 inclusive, but the average value for each of the different recovery times are found in Table 5. Because of wide variations in the minute values of CO_2 and O_2 obtained in the eight- and twenty-minute

recovery periods a considerable number of experiments had to be made before it was felt that the average was reliable.

In the five-minute recovery period the O_2 had returned to normal in the only two experiments which were run but the CO_2 had not quite reached the original level. In the eight-minute period the R.Q. of the second resting period was exactly the same as in the first period but both the CO_2 and O_2 remained a little lower, i.e., 6 and 5 cc per minute respectively. In the ten-minute recovery all five of the experiments done were consistent in showing a lowered O_2 absorption in the second period. The average reduction was 14 cc per minute. The decrease in the O_2 was still more pronounced when the recovery period was extended to fifteen

TABLE 5. SHOWING R.Q.'S AND CO_2/O_2 EXCHANGE FOR VARYING LENGTHS OF THE RECOVERY PERIOD
Subject E. T.

Work Period	Recovery Period	No. of Expts.	R. Q.				CO_2/O_2—cc/min.	
			Before Work	Work and Recovery	After Work	Excess Metab.	Before Work	After Work
5 min	5 min.	2	.86	.78	.90	.72	246/288	256/284
5 "	8 "	9	.86	.80	.86	.74	257/298	251/293
5 "	10 "	5	.86	.82	.89	.78	254/295	250/281
5 "	15 "	4	.86	.84	.87	.81	251/292	233/272
5 "	20 "	11	.89	.85	.85	.79	246/277	232/273
Weighted Average			.87	.83	.87	.77	251/288	242/281

minutes, namely 20 cc per minute. When the recovery period was continued for twenty minutes the results were quite irregular. Sometimes a large decrease and sometimes a large increase in both the CO_2 and O_2 values were found. There seems to be no other reason for this irregularity than the unrest occasioned by trying to sit so quietly on the bicycle for so long a time. The mouth piece and nose clip also make it rather uncomfortable for this length of time. However, the average of the experiments showed the O_2 to be practically the same but the CO_2 to be 14 cc less in the second recovery period. Because the second recovery period began at least thirty-five minutes after the first, we might well expect a slightly lower quotient in the second than in the first, which, indeed, is what is shown. From a consideration of all of the values obtained in these trial periods it seemed that a recovery period of eight minutes would be sufficient

for this subject doing the amount of work prescribed. Hill (17) notes that for moderate work six to eight minutes recovery is long enough for the oxygen to return to the true resting level.

The decrease in oxygen absorption following ten and fifteen minutes recovery is an interesting point and is to be found occasionally in the experiments of other workers. It appears that the oxygen debt (which in these experiments with very mild work is small and occasioned only by the lag in the response of the respiratory and circulatory systems at the beginning) is over paid, so to speak, so that there is a credit for a time and the oxygen absorbed consequently is less than the actual need. In other words a relative apnoea may follow the return to normal ventilation. This condition seems to prevail under the particular conditions of this experiment following a recovery of from 10 to 15 minutes but following a recovery of 20 minutes it has disappeared. (See Table 5.)

The Work Metabolism of Boys

In Table 4 we see that the average values for the first 37 experiments with subject E. T. showed the R.Q. for the second resting period to be exactly at the same level as it was at the beginning, 0.87. In the work and recovery period the quotient is 0.83, making the quotient of the excess metabolism 0.78. The average values for the CO_2 and O_2 for the second resting period indicate that the recovery had been complete.

No alveolar CO_2 determinations were made in this series but in the next eleven experiments, also listed in Table 4, the values obtained before work, after work and recovery, and after the second rest period showed very little change and the average values were practically identical. The second series of eleven experiments were made about seven months after the first series was completed. The results differ in several respects from those of the earlier experiments. The level of the resting R.Q. was higher by several points and the quotient of the excess metabolism was on the average ten points higher. In five of the experiments the quotient in the work period rose while in the preceding thirty-eight experiments this occurred only three times. The mechanical efficiency was higher in the second series, but curiously enough the recovery appeared to be longer. The subject during this time was leading a much more vigorous and active life than during the earlier experiments. In March of the preceding year he had received an injury which later became infected and gave him considerable trouble with the result that he had to discontinue most of his outside activities. He had spent the summer on a farm and returned in the fall in fine physical condition. He entered

TABLE 6. R.Q.'S AND CO_2/O_2 VALUES OF SUBJECT W. M.

Date	Expt.	Work (Cal.)	Work Period	Recov. Period	R.Q.				CO_2/O_2—cc/min.		Nutritive Condition
					Before Work	Work and Recov.	After Work	Excess Metab.	Before Work	After Work	
			min.	min.							
12– 5–25	1	6.85	10	10	.86	.83	—	.80	269/330	—	3 hrs. after breakfast
12–10–25	2	10.49	15	15	.83	.85	—	.86	270/324	—	4 hrs. after lunch
12–17–25	3	7.21	10	10	.81	.80	—	.79	271/334	—	4 hrs. after lunch
12–19–25	4	6.55	10	10	.83	.82	—	.80	299/362	—	1½ hrs. after breakfast
1– 7–26	5	6.38	10	10	.93	.89	—	.85	287/309	—	5 hrs. after lunch
1–26–26	6	8.09	15	15	.81	.88	—	.96	290/356	—	4 hrs. after lunch
2– 6–26	7	7.34	10	10	.93	.81	—	.71	278/299	—	3 hrs. after breakfast
2–22–26	8	3.45	5	7	.87	.79	.89	.69	301/345	282/317	3 hrs. after lunch
3–13–26	9	3.49	5	8	.91	.82	.84	.71	284/313	252/300	3 hrs. after breakfast
3–20–26	10	3.56	5	8	.94	.82	.87	.67	299/316	303/348	2 hrs. after breakfast
3–22–26	11	3.55	5	8	.84	.79	.84	.74	273/325	271/323	4 hrs. after lunch
3–27–26	12	7.15	10	10	.88	.78	.97	.69	302/344	303/312	1½ hrs. after breakfast
3–30–26	13	7.17	10	10	.85	.77	.90	.70	255/301	290/323	4 hrs. after lunch
4– 8–26	14	Det'n. of R.Q.'s only after breakfast									
4–29–26	15	3.41	5	8	.93	.88	.86	.79	328/345	297/345	4 hrs. after lunch
5–10–26	16	3.38	5	10	.81	.80	.90	.77	301/313	290/323	5 hrs. after lunch
5–12–26	17	3.62	5	10	.86	.87	.84	.88	287/333	273/323	4 hrs. after lunch
3–28–27	18	3.51	5	10	.87	.85	.94	.80	280/323	303/323	1½ hrs. after breakfast
Average					.87	.83	.88	.78	287/331	286/324	

into all the school athletics and Boy Scout activities and during the two months covered by the second series of experiments he was playing basket ball regularly. One might have expected that recovery from a slight piece of work such as he was doing on the bicycle ergometer would be more rapid when he was in better physical condition but such did not seem to be the case. The entire series of experiments on E. T. is long enough to smooth out the irregularities of individual observations and to make the average values significant. The R.Q. of the excess metabolism is certainly far from unity and in forty-one out of forty-nine experiments the work and recovery quotient was lower than the previous resting quotient, indicating, if any change, an increased combustion of fat.

In Table 6 are listed eighteen experiments made upon another boy, W. M. who likewise showed a fall in quotient during the work and recovery period in all but three of the experiments. The average fall was 0.09, making the excess R.Q. 0.78, the same as was obtained in the longer series with E. T. The average values for CO_2 and O_2 for the two resting periods in the last eleven of the eighteen experiments on W. M. indicated complete recovery and it is most probable that it was essentially complete in the first seven experiments although no determination in a second resting period was made.

For comparison with these two boys on normal diet we have sixteen experiments with an adult subject B. C. These are listed in Table 7. The subject was muscularly mature and in a good general state of training. The work varied from 4.48 Cals. to 13.7 Cals. Half of the experiments were of five minutes duration for work and from eight to twelve minutes duration for recovery. The other half varied from five to ten minutes for work and from eleven to seventeen minutes for recovery. The load was kept constant but the speed and time varied. In three of the experiments, Nos. 8, 9 and 13, in which the speed was 60 revolutions per minute, the R.Q. of the work and recovery period did not rise more than 0.02, but in nearly all the other experiments in which the speed was increased to 96 or 120 revolutions per minute the increase in the work quotient was greater. The average R.Q. of the excess metabolism was 0.95 and the average values for the CO_2 and O_2 indicated a nearly complete recovery. The alveolar CO_2 values at the end of the work and recovery period, however, were always higher than the previous resting values and averaged about 0.25 absolute per cent higher. (See Table 12.) With this subject, even with the most moderate work, which was but little more than that done by the boys E. T. and W. M., the R.Q. in the work and recovery

TABLE 7. NORMAL DIET
Subject B. C.

Date	Expt.	Work (Cal.)	Work	Recov.	R.Q.				CO_2/O_2—cc/min.		Nutritive Condition
					Before Work	Work and Recov.	After Work	Excess Metab.	Before Work	After Work	
			min.	min.							
2-12-27	8	4.48	5	8	.84	.86	.87	.88	178/212	182/209	Post Absortive
2-14-27	9	4.54	5	10	.86	.87	.90	.88	185/215	190/212	Post Absorptive
2-14-27	10	6.85	5	14	.84	.92	.84	.97	198/237	200/237	4½ hrs. after breakfast
2-15-27	11	6.92	5	12	.78	.86	.79	.92	195/249	196/249	3½ hrs. after breakfast
2-16-27	12	6.67	5	10	.82	.91	.83	.95	191/232	190/230	4½ hrs. after breakfast
2-17-27	13	4.52	5	8	.81	.81	.85	.81	173/216	193/226	Post Absorptive
2-17-27	14	6.85	5	10	.79	.87	.81	.93	186/236	190/236	5½ hrs. after breakfast
2-18-27	15	6.82	5	10	.79	.88	—	.93	175/221	—/219	Post Absorptive
2-18-27	16	10.29	7½	15	.78	.91	.82	1.01	205/264	206/252	5 hrs. after breakfast
2-19-27	17	13.70	10	17	.84	.90	.84	.94	180/216	203/242	Post Absorptive
2-21-27	18	13.70	10	18	.85	.95	—	1.01	186/219	—/246	Post Absorptive
2-22-27	19	7.52	5	12	.86	.98	.78	1.05	189/219	182/232	Post Absorptive
2-22-27	20	8.36	6	11	.85	.92	.77	.97	222/261	202/262	4½ hrs. after breakfast
2-24-27	21	11.10	8	15	.80	.91	.85	.97	191/239	207/243	Post Absorptive
2-25-27	22	11.10	8	15	.75	.87	.75	.94	170/229	180/241	Post Absorptive
2-26-27	24	11.20	8	15	.77	.87	.77	.93	117/231	188/241	Post Absorptive
Average		8.41			.82	.89	.82	.95	187/231	193/236	

period, rose slightly. It is probable, therefore, that this tendency is associated with the subject's physical condition and perhaps his state of maturity as compared with the boys'. No absolute basis of comparison was undertaken but the indications are that the boys did work at the expense of a larger combustion of fat while the mature subject did work at the expense of a larger combustion of carbohydrate. In order to see whether the nature of the diet was the determining factor, the following experiments were devised for the same subject, B. C.

Experiments on High Fat and High Carbohydrate Diets

For these experiments it was planned to supply a diet of about the usual caloric value with about the usual nitrogen content and then to interchange the fat and carbohydrate. The diet varied a little, usually in the amount of some foods, at the beginning of each one, but after the first day or two this was adjusted and the amounts listed below are the amounts maintained throughout the remainder of the period. The caloric value of each diet is shown in the following tabulation.

Carbohydrate Diet

Breakfast

75 gms. Cream of Wheat
50 gms. sugar
½ pt. skimmed milk
2 tbsp. lactose
½ glass orange juice
Coffee

Dinner

200 gms. lean meat
100 gms. cabbage
50 gms. cornstarch
50 gms. sugar
½ pt. skimmed milk
5 tbsp. maple syrup

Late Lunch

50 gms. cornstarch
5 tbsp. maple syrup

Fat Diet

Breakfast

50 gms. oatmeal
½ pt. XXXX cream
Coffee-saccharine

Dinner

200 gms. lean meat
100 gms. cabbage
25 gms. butter
70 gms. mayonnaise
¼ pt. XXXX cream
Diabetic Jello

Late Lunch

Diabetic Jello
¼ pt. XXXX cream

Approximate Caloric Value of Diets

Diet	Total Cal.	Cal. from Fat	Cal. from C.H.	Cal. from Prot.
Carboh.	2400	180	1920	300
Fat	2800	2200	340	260

The subject ate two meals a day at the laboratory and the food each time was carefully weighed. He came to the laboratory in the morning

without breakfast, an experiment was run, and breakfast was taken between 10 and 11.00 o'clock. Another run was made in the middle or late afternoon followed by dinner about 5.00 o'clock. The subject took home with him a weighed amount of food for a lunch later in the evening. (He had been in the habit previously of eating a light lunch before going to bed.) This was rather a fortunate arrangement as it gave one experiment in the post absorptive state and a second which was four or five hours after a meal.

Tables 8 to 12 contain the results of the special diet experiments on B. C. In all of these experiments the graphic record of the oxygen absorption was made and furnished a valuable guide for determining the length of time necessary to run the recovery period. The rate and depth of the respiration, as shown in the graphic record, was also taken into account and likewise the pulse rate. It may be well to note at this point that in some of the experiments, upon the fat diet expecially, recovery was not complete as judged by the O_2 absorption in the period following the work and recovery period. Nevertheless a study of the graphic record of oxygen absorption showed in several instances that it had come back to normal during the recovery period and had risen again in the second resting period, owing no doubt to the fatigue and discomfort from so long a time on the bicycle and from the mouthpiece and nose clip. Reference will be made to this point later.

In Table 8 are shown eight experiments on the high carbohydrate diet with eight minutes of work, averaging 11.21 Cals. for the entire period. The recovery period was from 16½ to 19 minutes in length which was sufficient. The initial average R.Q. was 0.83, the work and recovery quotient 0.92, the after period 0.80 and the excess quotient 0.96. Since the recovery on both O_2 and CO_2 was, on the average, complete, it is evident that more carbohydrate was burned during the work period than in the rest periods and indeed the excess quotient shows that practically all of the extra energy occasioned by the work was derived from this food stuff.

In Table 9 the first series of experiments on the fat diet is shown and we see that for the first four experiments the recovery period of 20 or 21 minutes was about right. In the following five experiments neither the CO_2 nor the O_2 had completely returned to the resting value even when recovery was continued as long as 28 minutes. However in the last experiment, which was only of 6-2/5 minutes duration, the recovery was complete. Apparently recovery is much slower on the fat diet as regards both CO_2 and O_2. As we shall see in the second series on high fat,

TABLE 8. CARBOHYDRATE DIET, FIRST SERIES
Subject B. C.

Date	Expt.	Work (Cal.)	Work	Recov.	R. Q.				CO_2/O_2—cc/min.		Nutritive Condition
					Before Work	Work and Recov.	After Work	Excess Metab.	Before Work	After Work	
1927			min.	mn.i							
2–28	25	11.20	8	17	.77	.94	.77	1.05	189/244	187/242	Post Absorptive
2–28	26	11.22	8	16½	.92	.90	—	.88	197/215	—	5 hrs. after breakfast
3– 1	27	11.22	8	16½	.75	.91	.77	1.00	177/237	180/233	Post Absorptive
3– 1	28	11.31	8	18	.78	.91	.76	1.00	192/247	192/251	6 hrs. after breakfast
3– 3	29	11.16	8	17	.85	.96	.86	1.01	173/204	184/212	Post Absorptive
3– 3	30	11.23	8	18	.91	.94	.81	.95	187/207	189/235	4 hrs. after breakfast
3– 4	31	11.15	8	18	.84	.89	.85	.91	175/207	176/206	Post Absorptive
3– 5	32	11.20	8	19	.89	.90	.81	.91	194/218	178/220	Post Absorptive
Average		11.21		17.5	.83	.92	.80	.96	185/223	183/228	

TABLE 9. FAT DIET, FIRST SERIES
Subject B. C.

Date	Expt.	Work (Cal.)	Work	Recov.	R. Q.				CO_2/C_x—cc/min.		Nutritive Condition
					Before Work	Work and Recov.	After Recov.	Excess Metab	Before Work	After Work	
1927			min.	min.							
3– 5	Started the diet										
3– 8	34	11.18	8	20	.80	.82	.79	.84	173/217	176/224	Post Absorptive
3– 8	35	10.17	7½	19¾	—	.82	.76	.86	—/250	189/248	4¾ hrs. after breakfast
3–10	36	11.08	8	20	.75	.81	.73	.85	177/237	173/237	Post Absorptive
3–10	37	11.11	8	21	.74	.79	.74	.83	198/252	193/260	4 hrs. after breakfast
3–13	38	11.19	8	25	.74	.77	.75	.79	172/233	192/256	Post Absorptive
3–14	39	11.20	8	22	.72	.79	.68	.82	171/237	179/265	Post Absorptive
3–14	40	11.16	8	23	.74	.78	.73	.81	175/238	199/270	5 hrs. after breakfast
3–15	41	10.40	7½	28	.70	.78	.77	.84	160/230	186/242	Post Absorptive
3–15	42	9.17	6¾	25	.73	.77	.74	.81	197/271	202/274	4 hrs. after breakfast
Average		10.85	7.7	22.6	.73	.79	.74	.83	175/230	187/253	

where the amount of work done was considerably less, the slower recovery on CO_2 is evident, but not on the oxygen.

When the subject was put back on the carbohydrate diet again the work still seemed hard as it had on the fat diet and after six experiments it was reduced from 1.4 Cals. per minute to 1.1 by slowing the rate of pedaling. This amount of work the subject was able to do satisfactorily but again it appears that the recovery was not quite complete, as judged by the CO_2 and O_2 values. (See Table 10.) However, the difference is not so large as it was on the fat diet and may be the result of fatigue from the position on the apparatus and not to anything peculiar to the diet. In the first part of this series the R.Q. started at 0.88, rose to 0.94 in the work and recovery period, and returned to 0.93 in the second rest period, making an excess R.Q. of 1.00. After the work was reduced the quotient started at 0.91, rose to 0.95, returned to 0.92 in the second rest period, making an excess metabolism of 0.99. The difference in height at which the initial quotient started in the second series on carbohydrate diet (Tables 8 and 10) was not due to differences in the diet but to differences in activity. During the first series the subject frequently danced or boxed in the evening thereby using up a considerable part of his glycogen; consequently he showed a relatively low R.Q. in the post-absorptive state the following morning. In the early part of the second series on carbohydrate there was apparently some hang-over effect of the fat diet immediately preceding. The subject felt "not so peppy as usual," to use his own expression, and the outside activities were still abandoned as they had been on the fat diet.

The last series of experiments on the fat diet again gave the most concordant results. (Table 11.) The initial R.Q. was low after the first day and fell gradually. The R.Q. for the work and recovery period was practically the same throughout and the excess R.Q. ranged from 0.79 to 0.82, making an average of 0.80. The R.Q. in the second rest period, however, was consistently higher than in the first rest period, due to higher CO_2. Recovery was complete in 25 or 26 minutes, so far as oxygen was concerned, but this period of recovery was not sufficient for a complete return to the average level of CO_2 elimination.

Table 12 shows the average values for the alveolar air, determined at the beginning of the first rest period, again at the end of that period, a third time at the end of the work and recovery period and a fourth time at the end of the second rest period. These alveolar air determinations are significant in several respects. On a normal diet and on the two carbohydrate diets the alveolar air at the end of the work and recovery

TABLE 10. CARBOHYDRATE DIET, SECOND SERIES
Subject B. C.

Date	Expt.	Work (Cal.)	Work	Recov.	R. Q.				CO_2/O_2—cc/min.		Nutritive Condition
					Before Work	Work and Recov.	After Work	Excess Metab.	Before Work	After Work	
1927			min.	min.							
3–17	43	11.19	8	22	.74	.88	.88	.99	174/235	205/232	Post Absorptive
3–17	44	11.15	8	23	.81	.92	.85	1.01	203/251	195/231	4 hrs. after breakfast
3–18	45	8.79	6½	21½	.90	.93	.92	.99	197/219	194/210	Post Absorptive
3–18	46	11.13	8	23	.98	.97	1.44	.96	223/229	223/215	4½ hrs. after breakfast
3–19	47	10.91	8	23	.78	.92	.91	1.02	474/224	204/224	Post Absorptive
3–21	48	11.10	8	22	1.06	1.04	1.00	1.02	210/189	201/200	Post Absorptive
Average		10.71			.88	.94	.93	1.00	195/224	204/219	
3–25	51	8.80	8	25	1.00	.95	.97	.92	217/215	213/220	Post Absorptive
3–25	52	8.79	8	23	.92	.97	.93	1.01	212/231	210/227	4 hrs. after breakfast
3–26	53	8.83	8	25	.79	.91	.85	1.03	183/233	201/237	Post Absorptive
3–27	54	8.79	8	25	.93	.96	.92	.99	190/205	217/235	Post Absorptive
Average		8.79		23.3	.91	.95	.92	.99	200/221	210/229	

TABLE 11. HIGH FAT DIET, SECOND SERIES
Subject B. C.

Date	Expt.	Work (Cal.)	Work	Recov.	R. Q.				CO_2/O_2—cc/min.		Nutritive Condition
					Before Work	Work and Recov.	After Work	Excess Metab.	Before Work	After Work	
3–29	Started Diet.		min.	min.							
3–30	58	8.79	8	25	.80	.81	.77	.82	181/228	175/229	Post Absorptive
3–31	59	8.71	8	25	.75	.77	.82	.79	174/231	195/238	Post Absorptive
3–31	60	8.82	8	25	.75	.77	.82	.79	180/241	196/239	5 hrs. after breakfast
4– 1	61	8.75	8	25	.74	.77	.80	.80	170/230	191/241	Post Absorptive
4– 1	62	8.74	8	23	.75	.77	.71	.79	181/242	202/250	Post Absorptive
4– 2	63	8.74	8	26	.73	.77	.83	.81	174/238	193/233	Post Absorptive
4– 3	64	8.78	8	25	.71	.77	.77	.82	174/244	183/239	Post Absorptive
4– 4	66	8.98	8	25	.72	.76	.83	.79	178/247	200/241	4 hrs. after breakfast
Average		8.80	8	25	.74	.77	.81	.80	176/237	192/238	

period was notably higher than at the end of the first rest period. Indeed this difference persists in these same series to the end of the second rest period. Evidently there was more CO_2 in circulation, therefore a higher tension in the alveolar air after the work than before. However, higher alveolar CO_2 tension does not of necessity denote a higher rate of elimination for, as shown in the minute values for CO_2 elimination, recovery was complete in the sense that CO_2 was being given off at the same rate in the second rest period as in the first. On the high fat diets a difference is noted. The alveolar CO_2 tension at the end of the work and recovery period in the first series was 0.31 absolute per cent higher than at the end of the first rest period but was not noticeably higher at this same time on the second high fat diet. Moreover in this first fat series the alveolar CO_2 tension fell rapidly following work in sharp contrast with its behavior on the high carbohydrate and normal diets. Urine tests showed the presence of aceto-acetic acid in both of these high fat diets. The alveolar airs also indicate a state of acidosis; consequently of more rapid elimination of CO_2 than was normal. As noted above at page 2, to make the

TABLE 12. AVERAGE ALVEOLAR CO_2 PER CENT

Subject B.C.

Diet	1 Before Basal	2 After Basal	3 After Work & Recovery	4 After 2nd Basal	Increase of 3 over 2.
Normal	5.20	5.31	5.56	5.54	0.25
Carbohydrate 1,	5.59	5.68	5.94	5.89	0.26
Fat 1,	5.03	5.18	5.49	5.29	0.31
Carbohydrate 2,	5.51	5.55	6.11	5.80	0.56
Fat 2,	5.21	5.33	5.35	5.32	0.02

evidence complete there should have been a determination of the CO_2-combining power of the blood. Nevertheless the evidence from the alveolar airs is what might have been expected from the combining power determinations. Because of the evidence of acidosis, mild though it was, the rise in R.Q. during work and recovery on the high fat diet can not be accepted as evidence of increasing carbohydrate combustion. Indeed we believe if the acidosis had been neutralized by administration of sodium bicarbonate the quotient would not have risen and might even have fallen.

The several series of experiments on special diets are summarized in Table 13. There is no doubt about increased carbohydrate combustion in this subject on high carbohydrate diet. The rise in quotient on the

high fat diet does not indicate, even taking the figures as they stand, the combustion of sufficient amount of carbohydrate to account for the work done and certainly in the presence of recognized acidosis the figures must probably be interpreted as indicating no increase in carbohydrate combustion whatsoever.

TABLE 13. SUMMARY OF AVERAGE RESULTS ON CARBOHYDRATE AND FAT DIETS
Subject B. C.

No. of Expts.	Work (Cal.)	Work	Recov.	R. Q.				CO_2/O_2—cc/min.		Diet
				Before Work	Work and Recov.	After Work	Excess Metab	Before Work	After Work	
		min.	min.							
8	11.21	8	17.5	.83	.92	.80	.96	185/223	183/22[illegible]	High Carbohydrate
9	10.75	7.6	22.6	.73	.79	.74	.83	175/230	187/253	High Fat
4	8.79	8	23.3	.91	.95	.92	.99	200/221	210/229	High Carbohydrate
8	8.80	8	25.0	.74	.77	.81	.80	176/237	192/238	High Fat

DISCUSSION

The average values obtained on all of the subjects who were upon normal diets have been collected in table 14 and we see that further observations have only confirmed the results obtained in the first few experiments, i. e., that the individual response to work is quite variable. In eleven subjects the quotient during work and recovery periods falls from the resting level; in some cases probably not beyond the limits of error, but in others quite beyond this. With two subjects it remained exactly the same and in five others it rose. If we consider that there has been no material change in the metabolism when the quotient showed a difference of only two points, we find that ten of the subjects exhibit this behavior with excess R.Q.'s ranging all the way from 0.77 to 0.96 depending upon the initial level, with an average of 0.86. Five showed a drop of more than two points giving excess R.Q.'s of 0.73, 0.74, 0.77, and 0.78, while three showed a rise giving excess R.Q.'s of 0.96, 0.99 and 1.00. This summary has taken into account the two separate series on E. T. and only the first series with the normal diet on B. C.

The factors of over-ventilation and recovery have been sufficiently discussed already. The results obtained, unless they have been affected by some other unrecognizable factor, justify the belief of Campbell, Douglas and Hobson (15) that "there is a good deal of difference between

different individuals and between the same individual at different times as regards the character of their metabolism" during work. In their experiments one subject (Hobson) did not change his metabolism appreciably during moderate work while the other (Douglas) always showed a rise in the R.Q. The authors suggest that the degree of physical fitness for the work in question may be of determining importance since Hobson was better developed and in a better state of training than was Douglas. However, in our own experiments E. T. gave a higher quotient when in the better physical condition and B. C. in still better physical condition gave the highest quotient of all even on a normal diet. Hill (17) has suggested that physical fitness for muscular work may mean amongst other things the capacity to store large quantities of glycogen and also, perhaps, the capacity to neutralize promptly excess quantities of lactic acid which remain unburned. On this interpretation a high quotient during muscular work would indicate a better state of training than a low quotient.

TABLE 14. AVERAGE R.Q.'S OBTAINED ON ALL SUBJECTS

		Respiratory Quotient				
Subject	No. Expts.	Before Work	Work & Recov.	After Work	Excess Metab.	Diet
M.W.	2	.82	.81	—	.79	Normal
T.B.	1	.94	.92	—	.90	"
R.D.	1	.82	.81	—	.79	"
J.P.	4	.84	.85	—	.90	"
C.M.	2	.85	.85	—	.85	"
P.G.	1	.93	.94	.86	.96	"
D.M.	1	.88	.88	—	.88	"
E.M.	2	.87	.81	—	.77	"
R.S.	4	.81	.78	.78	.73	"
J.J.	1	.81	.85	—	.99	"
B.K.	1	.89	.83	.88	.74	"
P.H.	2	.85	.88	.89	1.00	"
F.H.	2	.90	.88	.86	.86	"
J.L.	1	.82	.80	—	.77	"
E.T.	37	.87	.83	.87	.78	"
	11	.91	.89	.88	.88	"
W.M.	17	.87	.83	.88	.78	"
B.C.	16	.82	.89	.82	.95	"

A single experiment upon an individual cannot be considered as having much value. Similar results obtained upon ten individuals cannot be without significance. In ten of the seventeen subjects summarized in Table 14 it appears that the very mild piece of work provided did not

appreciably alter the nature of the metabolism. The body produced the necessary energy for the work by oxidation of the various materials in the same proportion as at rest. This is rather surprising in view of the fact that carbohydrate is presumed to be more easily oxidized and more readily available. Perhaps, however, the work must be of a certain severity to bring about a shift to the reserve supplies at least in some people. For very mild work the organism may find no diffculty in supplying the energy needed by simply increasing the combustion of the materials being presented to it at the time without putting any special call upon the glycogen stores. Perhaps a little more severe work would requisition these carbohydrate stores.

This is evidenced to some extent in the experiments with B. C. on normal diet in which the work was varied (see Table 7). When the work was 4.48 and 6.92 Cals. for the five minute period, the average excess R.Q. was 0.91. When it was between 7.52 and 11.10 Cals. and the time extended from 5 to 8 minutes the average excess R.Q. was 0.98 but the excess R.Q. obtained when the work reached the intensity of 13.7 Cals. for a ten minute period was no higher. With subject W. M. (Table 6) in the two experiments of fifteen minutes duration the R.Q. during work and recovery rose, a change which happened in only one other experiment with this subject and then only by a single point.

Confirmatory data is found in the work of Benedict and Cathcart (14) upon their subject M. A. M. In one series of experiments of moderate intensity the average R.Q. during work did not rise above the initial quotient but in the second series of greater intensity there was an average rise from 0.85 to 0.90. Douglas, Haldane, Henderson and Schneider (18) found that during walking a rise in the R.Q. could be detected when the rate of exercise was only sufficient to double the resting metabolism but that the rise became greater as the rate of walking increased. An increase in quotient during exercise can be seen also in the work of Benedict and Murschauser (19) on the metabolism of walking. Amar (20) obtained similar results in his experiments upon a bicycle ergometer and all of the observers have come to the conclusion that the increase in quotient probably is the result of increased combustion of carbohydrate. However, with very severe work we must bear in mind that the accumulation of lactic acid would tend to drive off CO_2 from the circulating blood and give a falsely high quotient. If we were to calculate the excess metabolism of all the experiments of the workers mentioned above, there is no doubt that many of them would give a quotient of about 1.0 indicating that carbohydrate alone furnishes the energy, but there is

likewise no doubt that many more would give a value considerably below 1.0 and probably little different from the previous resting value. With several subjects in our series there appears to be an increased oxidation of fat during work. W. M. showed a fall in quotient in 14 of the 17 experiments and E. T. showed a fall in 36 of the 49 experiments. With B. K. and E. M. the work quotient was six points below the initial level and with R. S. the average of four experiments was three points lower. In general it may be stated that the evidence from this study lends support to the belief of Zuntz that the body uses for the energy of muscular work both carbohydrate and fat and is in agreement with the recent results of Henderson and Haggard and of Himwich and Rose that the proportion in which these two food stuffs participate in the metabolism of work is practically the same as prevailed in the subject immediately previous to the work. There is but meager evidence in this study of an exclusive carbohydrate combustion during work even when the stores of glycogen in the body must have been more than sufficient to furnish the energy for the light piece of work which was done.

Summary and Conclusions

1. Experiments on seventeen subjects while riding the bicycle ergometer are reported. Most of the experiments were made on three subjects, two of them boys 14 and 15 years old respectively at the start and 15 and 16 at the conclusion of the experiments. The third subject was a young athlete 21 years of age, who gave instruction in boxing. He was well muscled and in good training generally but not especially trained for bicycle riding.

2. The ergometers used were of the Prony-brake type and the Krogh magnetic-brake type. Experiments on the same subject under like conditions gave equal efficiencies on the two ergometers.

3. In all the experiments on the athlete and in one series on one of the boys the nitrogen excretion in the urine was determined in a resting period and in a second period which included work and recovery and the post-work resting period. Alveolar CO_2 determinations were also made in these same series, after the preliminary rest, just before work, just after work and recovery, and at the end of the post-work resting period.

4. The R.Q. of the excess metabolism was obtained by deducting from the total CO_2 and O_2 of the work and recovery period, the CO_2 and O_2 obtained in the previous resting period.

5. Over 100 experiments were made upon subjects who were subsisting upon a normal (uncontrolled) diet.

6. A few experiments of the running-in-place type instead of the bicycle riding, were introduced for comparison.

7. Much time was spent in securing the proper length of recovery period so that complete recovery on CO_2 as well as on O_2 would be shown.

8. The work varied in intensity from 0.76 to 1.4 Cals. per minute and continued for from 5 to 20 minutes.

9. With one boy the average R.Q. of the excess metabolism occasioned by the work, in forty-one out of forty-nine experiments, was lower than the R.Q. of the previous resting period. With the second boy in 15 out of 18 experiments likewise the excess R.Q. was lowered. The average excess R.Q. with both boys on normal (uncontrolled) diet was 0.78. With the mature, athletic subject doing work of the same moderate intensity as the boys the R.Q. of the excess metabolism in 15 out of 16 experiments was higher than the R.Q. in the previous resting period giving an average of 0.95.

10. The athletic subject was placed on special high carbohydrate and high fat diets weighed carefully and consumed quantitively. On high fat recovery to the pre-work level of O_2 absorption and CO_2 elimination was much slower than on high carbohydrate.

11. The average excess R.Q. of work and recovery on both diets was higher than the R.Q. of the previous resting period, but the absolute level of the excess R.Q. very clearly was influenced by the diet. The alveolar values in these experiments on special diets showed: (a) more CO_2 in circulation at the end of work and recovery on high carbohydrate than previous to work; and (b) no change or less CO_2 in circulation at the same time on high fat. Urine tests showed the presence of aceto-acetic acid on the high fat diet. The presence of acidosis explains the higher R.Q. of the work metabolism on this diet.

12. In ten of the seventeen subjects studied the mild piece of work performed did not appreciably alter the metabolism. The body produced the necessary energy for the work by oxidation of the various materials in the same proportion as at rest.

13. In general the evidence from this study lends support to the belief of Zuntz that the body uses for the energy of muscular work both carbohydrate and fat.[1]

[1] Since the foregoing was written Rapport and Ralli (21) have published experiments on dogs which were made to run on a treadmill inside a respiration chamber. The dogs were maintained for two days prior to the work experiment on diets containing on the one hand 90% fat and 10% protein, and on the other 90% carbohydrate and 10% protein. They were studied in the post-absorptive state following these diets and the results were compared with those obtained

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in the same condition following normal diets made up of 55 to 60% carbohydrate, 25 to 30% fat and 15% protein. Due account having been taken of the oxygen recovery following periods of 45 minutes and 15 minutes of work, the excess metabolism of work and recovery over the pre-exercise basal was found to contain an oxidation of carbohydrate amounting to 61 and 55% respectively of the total on normal diet; 70 and 100% on high carbohydrate and 13 and 15% on high fat. The authors' conclusions coincide with our own.

Editoral Review

VITAL ECONOMY IN HUMAN FOOD PRODUCTION

THE early volumes of this Journal are dedicated to Lavoisier. A reproduction of his portrait by David appears on the cover. It was Lavoisier's discovery of the significance of oxygen in combustion, both inside the animal body and in the external world, which laid the corner stone of our present-day science of nutrition.

A short time before his untimely death upon the guillotine in 1794, Lavoisier was engaged upon a study of human calorimetry. He had previously devised an instrument for measuring with a fair degree of accuracy the heat produced in small animals, and had sought to balance this heat against the heat represented by the CO_2 and H_2O given off by the animal. It is not improbable that if his life had been spared for a few years longer he would have achieved (1) the demonstration accomplished only a century later by Rubner, of the law of the conservation of energy for the animal body. Rubner's achievement was perhaps the greatest contribution of all time on animal calorimetry. It stimulated immensely further studies in this direction, for it demonstrated a clear basis for the study of nutrition in the physical law established by Mayer and Helmholtz.

Rubner's experiments were made chiefly on dogs by the use of a calorimeter (2) which he described in 1894. The amount of heat given off to the calorimeter, measured by the expansion under constant pressure of a volume of air encased between the walls of the respiration chamber, was found to agree to within 0.3 of one per cent with the calculation of the heat produced, from the CO_2 elimination through the lungs and nitrogen through the urine and feces. Rubner's success stimulated the construction of a calorimeter by Atwater and Rosa, at Wesleyan University, Middletown, Conn., designed for similar experiments on man. This apparatus was first described (3) in 1897 and its first significant results were published in the same year. In addition to proving the appli-

(1) "Editorial reviews" will be a feature of this Journal. The term used implies that the review makes no pretense to completeness, but is a commentary intended to be authentic upon some important aspect of a subject with which the Journal is concerned. "Editorial" signifies not that the commentary necessarily is written by an editor, but that it has been read and criticized by at least four members of the Editorial Board.

cation to the human subject of the principles followed by Rubner in his study on dogs, Atwater and his associates demonstrated further that the energy consumed in muscular work, as computed from the products of respiration and protein metabolism, can all be accounted for by direct measurement of the heat discharged from the body.

It was the merit of the late Dr. H. P. Armsby, director of the Pennsylvania Institute of Animal Nutrition, at State College, that he saw the significance of these calorimetric studies for the vital economics of human food production in the bodies of farm animals; and it was through his insight that the Pennsylvania Agricultural Experiment Station and the Bureau of Animal Industry of the U. S. Department of Agriculture constructed a calorimeter at State College for the investigation of the energy exchange in cattle.

We take the occasion of the publication of this first number of the Journal of Nutrition to direct attention to the large significance of Dr. Armsby's work with this calorimeter, because of the relation of that work to the broadest considerations of national economy involved in the production of human food.

Work upon the apparatus was begun in the fall of 1898, the construction extending over nearly 3½ years. It was the first respiration calorimeter to be built for experimental work on the larger farm animals, and is at present the only apparatus of its type in existence.

Preliminary tests were completed, and the first actual experimental work was begun early in the year 1902. The apparatus and methods used are described by Armsby in Bulletin 104 of the Pennsylvania Agricultural Experiment Station.

The chief object of the first of Armsby's investigations was to test Rubner's theory, promulgated in 1883, of isodynamic replacement (4) of nutrients. As applied to animal nutrition this theory stated that "in amounts less than a maintenance ration, the several organic nutrients replace each other in proportion to their metabolizable energy." Through the investigations of Zuntz and others it had been shown that there was a considerable expenditure of energy in the digestion and assimilation of food, which had not been taken into account by Rubner. Rubner himself later was led to abandon this theory of isodynamic replacement, but the insecurity of its foundation in relation to ruminant animals in particular was made evident when the results of the early experiments of Armsby and Fries (5) were published. Rubner's theory presupposed that foods influenced the heat production equally, if at all, but the experiments of Armsby and Fries on the available energy of timothy hay,

published in 1903, showed that the energy cost of digestion and assimilation of the feed was so great that the heat produced in the animal, *at or even below the maintenance level* of nutrition, was far in excess of the amount ordinarily needed for maintenance of body temperature.

Rubner meantime was engaged on his studies of heat regulation which led finally to the formulation of his laws regarding the specific dynamic action of foods. These laws, as is now well known, demonstrated conclusively that when the animal was placed under conditions where the influence of environing temperature was minimal, each of the three organic foodstuffs exerted a peculiar and specific effect on the heat production, protein producing, *in 24-hour periods*, the largest increment of heat, fat next, and carbohydrate least. Armsby's results confirmed for the ruminating animal the existence of an even larger factor of what might be called waste heat, which, as he demonstrated, must be taken into account in evaluating different foods for animal production.

Guided by the results of his early calorimetric researches with cattle, and also by the results obtained by Kellner (6) in Germany by means of indirect calorimetry, Armsby devised a method of determination and statement of the nutritive value of feeds in terms of energy, wherein all losses and expenses of feed utilization, including the loss in the form of heat, are accounted for. These values represent, as he intended, the net effect of feeds on the animal and were termed by him "net-energy" values (7). This conception of net energy constitutes one of Armsby's greatest contributions to the science of animal nutrition. The importance of this conception, and its practical bearings on the problems of animal nutrition, are discussed by Forbes and Kriss in Chap. XIV of "Chemistry in Agriculture" (8).

One of the most intricate questions that asserted itself at an early stage of Armsby's work at the Pennsylvania Institute was the question of the relative utilization of the metabolizable energy for maintenance on the one hand and for production of body increase on the other. By metabolizable energy of the feed of the ruminant animal is meant the gross energy of the feed minus that of the urine and feces and of the methane gas produced by fermentation. Armsby asked himself whether the rate of utilization of this metabolizable energy is the same at all planes of nutrition. In other words, does food have the same net-energy value when fed in amounts below the requirement for maintenance of live weight as when fed in amounts sufficient to produce new tissue and fat? Armsby, after some justifiable fluctuations of opinion, reached in 1918 a tentative answer to this problem in the affirmative—a conclusion later found to

be erroneous when the directly determined fasting metabolism was adopted as a measure of the maintenance requirement.

Armsby was succeeded in the directorship of the Pennsylvania Institute of Animal Nutrition by Dr. E. B. Forbes, who since his appointment has been loyally and industriously pushing the questions propounded by his predecessor, as well as other problems, to solution. Of Armsby's conception of net energy he has stated recently that, "it is the simplest and most inclusive of all general measures of nutritive value." "Net energy is the remainder after the deduction of all expenses and losses of utilization from the gross income of energy." More specifically, the net energy of a feed is found by subtracting from the total heat of combustion, that is, the gross energy, the energy equivalent of the feces and urine, the methane and the heat produced by fermentation of carbohydrates in the alimentary tract, also the energy represented by the increased heat production caused by the physical work of feed utilization, and finally the augmenting effects upon energy metabolism of the nutrients after their absorption. This last factor is called in human nutrition the specific dynamic action.

One of the problems which has engaged the attention of the Institute in recent years is the determination of the net-energy value of the feed for the production of milk as compared with its net-energy value for the production of meat on the hoof in the same animal. Several papers (9, 13,) have been published from the Institute bearing upon this question.

Before this problem could be satisfactorily solved it was necessary to establish a basis for the determination of the maintenance requirement of the animal for net energy. Armsby's method (10) had been to find this maintenance requirement by extrapolation from the amounts of energy produced at different levels of nutrition. For example, if an animal fed one-half more than the amount of food necessary to maintain its weight, produced a certain amount of heat which we may call A, and at twice the maintenance level produced an amount which we may call B, projection of the line connecting B and A backward to the no-feed base line would give a heat production considered by Armsby to represent the maintenance requirement. The reason why the heat production *at the maintenance level* of nutrition cannot be considered as a measure of the net energy requirement of maintenance is that when only enough food is given to maintain the animal in theoretical equilibrium, a considerable amount of this food energy is wasted through the dynamic effect, and other expenses.

Rubner had used the fasting metabolism of dogs as a base value in determining the specific dynamic action of foods and Armsby likewise

considered the fasting metabolism to be the correct measure (11) of the maintenance requirement of net energy. While he had always considered it impracticable to make a satisfactory, direct determination of the fasting metabolism of cattle and to the time of his death employed the above method of computation which he had devised, he nevertheless outlined an experiment in 1920 which should give the answer, "whether it is possible to determine the basal katabolism" directly. The work of the Institute on the subject of lactation, begun under Armsby and continued under Forbes, first made possible this comparison of the computed and the directly determined fasting metabolism (12) and showed that direct determinations are entirely practicable and give values which are not only less variable but also much higher than those obtained by the computation method.

In the Institute's studies of lactation five series of experiments with cows in either the lactating or the dry condition were conducted during the years 1916, 1920, 1921, 1922 and 1925, these experiments dealing with the efficiency of the milch cow in terms of energy to utilize feedstuffs, especially for milk production in comparison with maintenance and with body gain.

Referring to the last four series of studies, the heat production and gains in energy were determined by direct as well as by indirect calorimetry. The maintenance requirement of net energy was directly observed with each cow while fasting and the efficiency of utilization of feed energy for body gain was determined for each cow while she was dry. From the data thus accumulated it was possible to calculate the net-energy value of food for milk production as compared with the net-energy value of the same for body gain. These experiments are so important from the standpoint of vital economy of food production for human purposes in general that we shall describe them in some detail.

Three cows were chosen, two of them pure-bred Jerseys and another of the same breed but of unknown pedigree. Each experimental period covered 21 days, the first eleven being preliminary to accustom the animal to the ration and to all the other conditions imposed by the experiment, and the last 10 days constituting the collection period. In this period the milk, urine and feces were collected and sampled for chemical analysis. During the last two days of the collection period the animal was subjected to the respiration calorimetric study, in which the heat production, CO_2, water vapor, and methane were measured.

With two of the animals two series of observations were made, one as early and one as late as practicable in the period of lactation. Both

animals were kept on a plane of nutrition estimated to be slightly above the requirements for maintenance and milk production but not permitting of fattening to any significant extent. The third animal was used to determine, in conjunction with the study of utilization of food energy, the effects on milk production of different quantities of the same feed. For this four periods were used. During the first and fourth the cow was given as much feed as she would eat without waste. In the second period the ration was computed to nearly the maintenance requirement, and in the third period the ration was increased by half the difference between those of the first and second periods.

The standard ration used throughout all the experiments consisted of alfalfa hay, cut in 1-inch lengths, and a grain mixture composed of 10% linseed meal, 30% wheat bran, 30% ground oats and 30% corn (maize) meal, all of good quality. The ratio of hay to grain throughout all the experiments was 2 to 3 in weight. Common salt was fed in the amount of 30 grams per day, 15 grams in each feeding.

Without going into details of the analytical procedures, we give some of the results which illustrate what may be accomplished by animal calorimetry in this important field. The agreement between the heat production as computed from the excreta, with due allowance for the balance with respect to fat and protein, and for the milk production on the one hand, and the heat as measured by the calorimeter on the other, is remarkably good, showing but little more than 1% difference in several of the experiments. We present in Table 1, copied from one of the Institute's publications (13), the energy of the milk produced, the energy of

TABLE I. ENERGY OF MILK PRODUCED AND OF BODY GAIN, BASED ON THE OBSERVED HEAT PRODUCTION OF COWS

Exp. No.	Cow No.	Period No.	Dry Matter in feed eaten	Metabolizable Energy: Per kgm. of dry matter of feed	Metabolizable Energy: Total	Obser. Heat Prod.	Energy of Milk	Gain or loss of energy by body
			Kgm.	Cals.	Cals.	Cals.	Cals.	Cals.
221E	874	I	6.879	2,462	16,934	11,417	4,931	+586
	886	I	8.105	2,479	20,090	12,048	6,498	+1,544
		II	6.990	2,572	17,978	11,049	5,162	+1,767
221F	886	II	8.067	2,571	20,741	12,422	5,935	+2,384
221G	887	I	8.033	2,575	20,686	12,368	7,947	+371
		II	3.846	2,437	9,374	8,627	5,589	−4,842
		III	5.935	2,566	15,229	9,870	5,648	−289
		IV	8.027	2,528	20,296	11,670	6,045	+2,581

the food metabolized, and the energy stored in the body or lost from its substance, according as the animal was above or below energy equilibrium. In this table it will be noted that metabolizable energy minus the observed heat production by direct calorimentry, and minus also the energy of the milk produced, gives the gain or loss of energy by the body.

Some extremely interesting points in the economy of milk production are illustrated by this table. The variations in milk production do not appear to follow so closely the variations in energy absorbed as might be expected. In other words, milk production is so largely a matter of individual capacity as to appear more or less independent of food supply, and yet with cow No. 887 a sharp reduction of energy intake produced a marked fall in milk production, and in addition a heavy drain upon body tissues, as shown by the large negative balance of energy. Adding about 6000 Cals. of metabolizable energy in the third period of experimentation (as compared with the second) with this cow had little effect on the milk production, but restored almost to equilibrium the energy exchange of the body itself.

Again a sharp difference is noted between the first and fourth period with this cow on the same plane of nutrition. This difference might be accounted for by the fact that the first period occurred early in lactation and the last period late in lactation, or at least much later. The intake of metabolizable energy in the two periods differed by only 400 Cals. in a total of over 20,000. The milk production in the earlier period, however, contained nearly 2000 Cals. more than in the last, and as a consequence of this difference the energy gained by the body in the last period was quite 2000 Cals. more than in the first. We may conclude that the stage of lactation is a determining factor in the disposition of the metabolizable energy. However, it is a matter of well substantiated observation also that underfeeding, as in the second and third periods, renders it very difficult, if not impossible, to bring a cow back to her earlier rate of milk production by the use of any amount of feed.

Many additional points of interest might be pointed out in this study of lactation. We must content ourselves here with drawing attention to the summary which compares the net-energy value of the feed, on the basis of one kilogram of dry matter for the several purposes to which energy may be put in the lactating animal; namely, body maintenance, body increase, and milk production.

The value of the net energy of the feed for the maintenance of a cow is derived by the following simple calculation: The directly determined fasting metabolism is expressed in terms of Calories per square meter

of body surface. This value multiplied by the number of square meters of the body surface of the cow during periods of feeding, gives the Calories of net energy required for maintenance during these periods. This amount subtracted from the total heat production of the animal on the maintenance diet, while in the dry condition (9) gives the total heat increment of the ration. The remainder of the metabolizable energy expressed as Calories per kilogram dry matter of the feed, gives the net-energy value of the feed on the same basis. By similar calculations based on determinations made while the cows were dry but gaining in weight and others while they were giving milk, Forbes and his colleagues have been able to assess seemingly correct relative values of feed energy for purposes of maintenance, body increase, and milk production. These several values are compared in the following tabulation.

TABLE 2. RELATIVE VALUE OF FEED ENERGY FOR MAINTENANCE, BODY INCREASE AND MILK PRODUCTION

	NET ENERGY VALUE PER KILO OF DRY MATTER OF FEED			UTILIZATION OF FEED ENERGY		
Cow No.	For Maintenance	For Body Increase	For Milk Prod.	For Maintenance Standard	For Body increase (relation to maintenance).	For Milk Prod. (relation to maintenance).
874	1,863	1,520	1,842	1	0.816	0.989
886	2,003	1,396	1,881	1	.697	.939
887	1,871	1,438	1,924	1	.769	1.028
Aver.	1,912	1,451	1,882	1	.761	.985

The interpretation which the authors place upon these results is as follows: "The average rates of utilization of the net energy of the ration for maintenance, lactation and body increase were found to be as 1 for maintenance, 0.985 for lactation and 0.761 for body increase. More extensive evidence will doubtless modify this apparent relationship; but accepting the same provisionally, it is certainly of interest and consistent from a teleological point of view, that with a lactating female the rate of efficiency of utilization of food for the maintenance of the life of the mother and for the production of milk for the offspring are apparently alike, while the economy of use for body growth is at a distinctly lower rate."

That the efficiency of utilization of energy for milk production is essentially the same as its efficiency for maintenance appears consistent,

if it be considered that energy used in milk production is employed as in maintenance for the prevention of body loss, the milk in this relation being regarded as body substance.

In the light of current conceptions regarding the causes of the specific dynamic action of foods, we may understand that the synthesis of proteins from amino acids for production of milk, and the consequent removal of stimuli (14) or catalyzers (15) of oxidation, takes place at much the same rate as it does in ordinary repair of tissue, other things (rate of absorption, kind of amino acids, etc.) being the same, but at a more rapid rate than in growth or fattening. In the latter condition plethora of carbohydrate and fat doubtless would play a rôle also in producing higher dynamic effects and therefore lower net energy of the food.

At all events it is clear that the utilization of food for the production of milk in the Jersey cow is about 30% (0. 224÷0.761, See Table 2) more economical, in the physiological sense, than the production of body increase in the same animal.

The significance of these facts has been realized on a national scale, as for example in Denmark and other countries during the recent war; and it will be recalled that the war stimulated the production of milk at the expense of meat production even in the United States which is not yet obliged rigidly to husband agricultural resources.

Whether the conclusions reached by Forbes and his co-workers can be applied generally to cattle of all breeds remains to be determined by further investigation. Considering, however, the broader uses to which milk may be put as compared with meat in national nutrition, the findings here discussed cannot fail to be of interest to those who regard the subject of nutrition from the national as distinguished from the individual point of view.

Other very highly instructive results for the student of human nutrition no less than for the student of animal nutrition, which have been contributed by the Pennsylvania Institute in recent years, relate to the influence of environing temperature on the maintenance requirement of energy; to the effect of fatness on the economy of food utilization; and to the plane of nutrition as affecting energy metabolism.

In all warm-blooded animals, including cattle, the mechanism of heat regulation is so adjusted as to maintain an approximately constant body temperature. This heat comes from the food, but its dissipation is so regulated by the mechanics of the blood vessels and sweat glands that under ordinary circumstances just as much heat is lost as is produced. In other words, the body is a thermostat like a laboratory incubator

with its regulating mechanism set, for example, at 98.6° F. as in man. When, however, the environmental conditions are such as to induce a more rapid loss of heat from the animal body than is produced as a consequence of the physical and chemical activities prevailing, then the animal must increase its oxidation of food or body nutrients in order to maintain the normal body temperature. This is Rubner's "chemical regulation." The temperature of the surrounding air below which the chemical regulation is brought into play is called the critical temperature. It exists for man as well as for farm animals, although it may not "trip the mechanism" at the same level on account of different provisions which nature has made or which man has adopted to guard against heat loss.

Suppose one is sitting in a cold room and begins to shiver. Shivering is for the purpose of increasing heat production in order to maintain the normal body temperature. But if a man doesn't enjoy shivering, he may raise the room temperature, or put on more clothing, or exercise, or take food, if he hasn't recently done so. Any of these measures if sufficient will counteract the loss of heat or prevent it and restore equilibrium. The farm animal is provided in the winter with a heavier coat of hair, and on the average is fatter and eats more food than in the summer. The critical temperature consequently is lower than in summer time. In other words, the animal can withstand lower air temperatures without calling upon the body resources directly for heat production.

It is obvious that in studies of the effect of food on the heat production of farm animals for net-energy values, it is necessary to guard against subcritical temperatures lest the resulting increase of heat production be erroneously regarded as a part of the energy expense of food utilization.

As an instance of errors which may arise from neglect of this factor we have the example of Rubner's law of isodynamic replacement. Rubner had conducted the experiments upon which this theory was based at temperatures below the critical for his animals; but he was keen enough to appreciate the importance of external temperature, and when he later repeated his experiments at temperatures well above the critical he discovered his laws of specific dynamic action, and therefore abandoned his theory of isodynamic replacement.

Experiments (16) at the Pennsylvania Institute demonstrated that a steer weighing 335 kgms. would increase his heat production 36 Cals. per square meter of body surface for each degree Centigrade of the environing temperature below the critical level—or, in the normal fasting state, about 2½% increase for each degree below the critical.

How long does it pay to feed steers in the process of fattening? Does

the already fat animal make as economical use of the food as an animal which is thin to start with? This has been a moot question amongst animal producers and agricultural economists. It was found by Armsby and Fries (17) in actual experiments upon fat and thin cattle that feeds were digested equally well by both, and that the heat loss resulting from the consumption of feed (what we would call the specific dynamic action in man) was a little greater with the fat than with the thin animal. Consequently the net-energy value of the feed was slightly less with the fat than with the thin animal. However, the maintenance requirement for the fat animal of the same size was materially increased. This greater maintenance requirement together with a relatively somewhat smaller feed consumption in the already fattened animal was chiefly responsible for the greater cost of the gains produced.

One of the latest studies (18) of the Institute is an analysis of the influence of the plane of nutrition on the energy metabolism—a subject of especial importance in relation to the methods of determination of net-energy values of feeds for maintenance and for body increase.

The subjects were two Aberdeen-Angus steers, between 25 and 33 months of age; and throughout the study the results with the two steers agreed phenomenally well. They were studied at each of five planes of nutrition. As indicated by the quantity given, these planes were (1) fasting, (2) half of the maintenance requirement, (3) maintenance (energy equilibrium), (4) half more than maintenance, and (5) twice the maintenance requirement. The rations were composed of corn meal and alfalfa hay in equal parts.

The heat production increased only a little from the fasting plane to the plane of half of the maintenance requirement, but increased much more rapidly from the latter plane of nutrition to the maintenance level. However the entire rise in metabolism from the fasting to the maintenance level was at a distinctly lower rate than was the fairly uniform increase from the level of maintenance to that of twice the maintenance requirement. The relation between the heat production and the dry matter of the food, above maintenance, is expressed by a nearly straight line, but below maintenance by a pronounced curve, as shown in the illustration.

The several factors which determined the curve of heat production in relation to the dry matter of the feed seem to be, (1) the amount of the observed heat production of fasting (as the base value), (2) the energy cost of utilization of food, (3) the difference in the energy cost of utilization of food for body increase as compared with the utilization of the same for

the prevention of body loss, and (4) the conditions affecting the digestibility and metabolizability of the food at the different planes of nutrition, since, because of these factors, the quantities of nutrient actually available are not quite proportional to the amounts of food consumed.

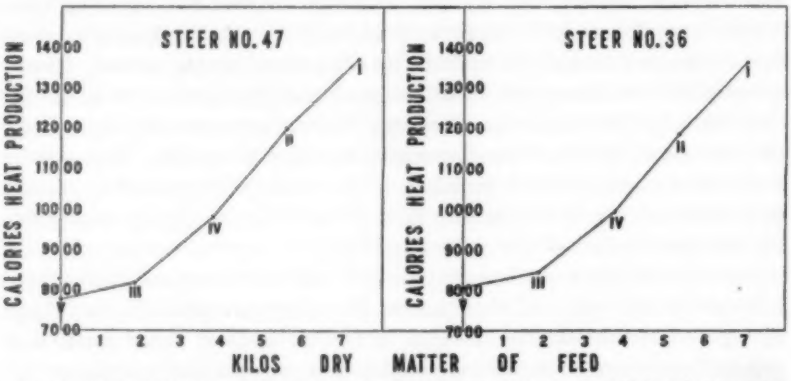

FIG. 1. Heat Production as Related to the Dry Matter of Feed. I Full feed (twice maintenance), II Half more than maintenance; III Half maintenance; IV Maintenance (energy equilibrium); V Fasting.

The character of the curve representing heat production below the plane of energy equilibrium suggests a specific dynamic effect of body protein and fat mobilized during fasting. The true maintenance requirement for net energy would, according to this view, differ from the observed heat production in fasting by the amount of this endogenous specific dynamic effect, could it be determined.

The authors have worked out also curves, for which we have no space, representing the distribution of feed energy per kilogram of dry matter, between feces, urine, methane, and heat increment, and digestible, metabolizable, and net energy, as affected by the plane of nutrition. From the plane of half of the maintenance, to that of twice the maintenance requirement, these curves were characterized as follows: Digestible energy first rose slightly, on account of increased digestion of crude fiber, and then decreased at an increasing rate, as a result of lowered digestion of carbohydrate and protein; the curve of energy of the feces, naturally, was the reverse of that of digestible energy; the curves of methane and urine energy were nearly straight lines, falling slightly with rise in plane of nutrition; the curve of total heat increment (computed with reference to the fasting metabolism) rose at each period of observation, but each time at a decreased rate of rise.

The curve of total net energy (for maintenance and body increase together) was a practically straight line, falling rapidly with rise in plane of nutrition; but these total net-energy values are of mixed significance since each represents a different status of the animal with reference to energy balance.

The net-energy values of the ration, with the two steers, were, for maintenance, 2218 and 2241 Calories; for body increase at half more than maintenance 1550 and 1557 Calories; and for body increase at twice the maintenance requirement, 1531 and 1557 Calories per kilo of dry matter.

The results of this study make it clear that net energy values of feeds for increase of body weight in the steer are lower than are such values for maintenance of body weight. If a listing of net energy values of feeds is to be of any value for use in the practical production of meat on the hoof, these two values must be separately stated, or a compromise between them be clearly recognized. A particular service rendered to the broader science of nutrition by this study is the demonstration that comparable determinations of the specific dynamic action of different foods can be made only at the same plane of nutrition.

In conclusion it would not be too much to say that the torch lighted by Lavoisier and brightened by the intellect of Rubner has been carried forward creditably in the work of the Pennsylvania Institute to illuminate important economic problems of nutrition.

J. R. M.

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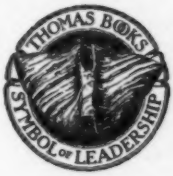

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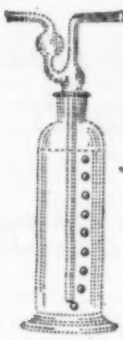

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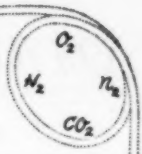

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